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STUDIES IN CALCIUM AND PHOSPHORUS METABOLISM*

XI. THE CALCULATION OF ACID BASE CONTENT OF THE DIET

By

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THE estimation of the acid base balance in diets is becoming progressively more important in metabolic studies as well as in the treatment of disease. It is obvious that in the study of electrolyte metabolism there are several important factors—the intake, the absorption from the intestinal tract, and the effect of absorption upon the excretion both of recently ingested and of previously stored electrolytes. Thus it is important to determine quantitatively the effect of the ingestion of excess acids upon the excretion of bases such as calcium, which are stored in the body.

A series of experiments in such problems was undertaken in the metabolic ward previously described by Bauer and Aub (1). The results of these observations on the electrolyte metabolism of man are reported elsewhere, but a discussion of the difficulties involved in the accurate dietetic management of these investigations is the purpose of this communication. In these observations the attempt was made to vary the potential acidity[1] or alkalinity of the diet without altering the total low calcium intake. The calcium and ammonia excretion then afforded an accurate method of determining the influence of varying procedures upon the excretion of fixed electrolytes stored in the body.

In 1912 Sherman and Gettler (2) called attention to the importance of inorganic constituents of the diet as sources of the excess acid or base excreted, and as important factors influencing the amount of urinary am-

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[1] Potential acidity may be briefly defined as the excess acid (referred to the hydrogen ion concentration of the blood) of the inorganic residuum of the diet after oxidation.

monia. Tables of analyses for many foods have since appeared in Sherman's book from which the excess acid or base content of the common articles of the dietary may be estimated.

Subsequently, Blatherwick (3) confirmed Sherman's deductions, that, with the exception of certain fruits yielding organic acids, the alkalinity or acidity of the urine is normally determined by the inorganic constituents of the food. The relation between acid excretion and calcium excretion has been discussed by Aub (4) and associates (5).

For the purpose of investigating accurately the excretion of inorganic electrolytes on diets of known composition, it is necessary to control the potential acidity of the food in order to arrive at sufficiently accurate standard levels of excretion. If the inorganic constituents of a diet are known quantitatively and if it be assumed that no considerable amount of organic acid escapes oxidation, it is possible to calculate approximately the potential acidity or alkalinity of a given dietary. The calculation of the potential acidity of a diet can be made if the inorganic base-forming elements, *i.e.* Ca, Mg, Na, and K, be summated in terms of N alkali and the inorganic acid-forming elements, *i.e.* Cl, S, P, be summated in terms of N acid.[2] The difference between these two sums gives the potential acidity or alkalinity of the diet, barring the excretion of much organic acid. That this calculation cannot be made with great accuracy, even when food is accurately weighed, is apparent not only from consideration of possible natural daily variations in the composition of foodstuffs, but also from the difference in values to be found in the literature for individual foods. Most of the estimations of the inorganic constituents of the foodstuffs used have been based on tables compiled by Sherman (6). If the data and discussion which follow indicate discrepancies between estimated values and actual analyses of our diets, they should in no sense be interpreted as unfavorable criticism of Sherman's very valuable work. These differences do not detract from the value of such compilations for use in routine dietetics. It is our purpose merely to examine the reliability of estimated potential acidity data for accurate metabolic experiments in the clinic. In some instances the earlier values published in 1911 by Sherman, and long used by dietitians, are given for comparison with his more recent estimates to emphasize the necessity of using up-to-date tables in dietary computations.

The following estimate for meat, based on Sherman's 1927 figures for meat protein (see Table I), gives an excess acid of 16.5 cc. N acid per 100

[2] Sulfur is reckoned as divalent; and the valence of phosphorus considered to be approximately 2.

grams. His 1911 figures, however, yield values for several different samples of meat, as follows: 13.91, 10.05, 12.00, and 13.67 cc. N (averaging 12.4 cc. N. acid).

TABLE I

POTENTIAL ACIDITY OF MEAT

Meat (21.3% protein)	Ca		Mg	K	Na	P		Cl	S
Total, gm.	.012	*.008*	0.025	0.361	0.090	0.230	*0.172*	0.081	0.244
Total, cc. N	.62	*.40*	2.1	9.2	3.9	14.8	*11.1*	2.3	15.2

Total cc. N acid	32.3	*28.6*
Total cc. N base	15.8	*15.6*
Total cc. N excess acid	16.5	*13.0*

Note: Supplementary analyses from Massachusetts General Hospital of Ca & P are indicated by italics. Other values are taken from Sherman, 1927.

Table II shows that the difference between Sherman's estimate of excess base in apples in 1911 and 1927 is negligible.

TABLE II

APPLE, ACCORDING TO SHERMAN (per 100 grams)

	Ca		Mg	K		Na		P		Cl		S	
Total, gm.	.007	*.010*	.008	0.127	*0.125*	.011	*.015*	.012	*.013*	.005	*.004*	.006	*.005*
Total, cc. N	.4	*.5*	.7	3.3	*3.2*	.5	*.7*	.8	*.8*	.1	*.1*	.4	*.3*

Total cc. N base	4.9	*5.1*
Total cc. N acid	1.3	*1.2*
Total cc. N excess base	3.6	*3.9*

Note: Figures in italics according to Sherman, 1911. Other figures according to Sherman, 1927.

In Table III, however, is given a similar estimate for banana calculated from Sherman's 1927 figures. The 1911 estimate (in italics) was 5.8 cc. N base per 100 grams edible portion as compared with 8.5 in 1927.

Analyses of several foods for calcium and phosphorus content have been made in this laboratory, some of which are compared (in Table IV)[3] with the corresponding values given by Sherman. Substitution of these values for Sherman's will yield a different acid base value.

[3] A more complete table of analyses made in this laboratory appeared in the *Journal of the American Dietetic Association*, Vol. III, No. 2, September, 1927. The phosphorus determinations are open to the objection that the samples were ashed in a furnace. Inasmuch as the results are frequently higher than Sherman's figures, however, the objection would appear not to be consistently valid.

TABLE III
BANANA, ACCORDING TO SHERMAN
(per 100 grams)

	Ca		Mg		K		Na		P		Cl		S	
Total, gm.	0.009	*.007*	0.028	*0.024*	0.401	*0.415*	0.034	*0.015*	0.031	*0.024*	0.125	*0.200*	0.010	*0.013*
Total, cc. N	.5	*.4*	2.3	*2.0*	10.3	*10.6*	1.5	*.7*	2.0	*1.5*	3.5	*5.6*	.6	*.8*

Total, cc. N base	14.6	*13.7*
" " " acid	6.1	*7.9*
" " " excess base	8.5	*5.8*

Note: Figures in italics according to Sherman, 1911. Other figures according to Sherman, 1927.

In Table V are given the acid base values for a few foodstuffs, calculated according to Sherman's figures and again with values for Ca and P from this laboratory substituted for his 1927 figures. An extreme example is found in chicken, which, according to Sherman (1911), contained, per 100 grams, excess acid amounting to 17.0 cc. N acid, but according to our analysis only 10.3 cc. N acid. Inasmuch as chicken may constitute an important part of a high acid or high protein diet, the discrepancy between these estimates is of considerable importance.

Another food for which it is rather difficult to evaluate the acid base content is cooked bacon, due to the fact that in the process of cooking much fat separates from the food, leaving behind a high salt content relative to the percentage weight. The difference in the potential acidity of bacon, before and after cooking, is illustrated by Table VI. The estimates for cooked bacon are based on analyses for Ca and P performed in the Massachusetts General Hospital. The NaCl content was determined by Miss Phoebe E. Drury at the Collis P. Huntington Memorial Hospital by Van Slyke's method. The protein content was taken as 23 per cent (Sherman, 1927, cooked bacon). Other figures are fixed as twice those for the mineral content of raw bacon.

The discrepancy in total electrolyte content is of course far greater than the difference between the values for potential acidity. Nevertheless, for equal

TABLE IV

SHERMAN'S CA AND P FIGURES COMPARED WITH M.G.H.* ANALYSES

	Calcium (per 100 grams of edible portion)		Phosphorus	
	Sherman	M.G.H.	Sherman	M.G.H.
Apple	.007	.010	.012	.018
Banana	.009	.007	.031	.042
Corn	.006	.005	.103	.061
Steak	.012	.008	.229	.172
Chicken	.011 (1911)	.018	.258	.162
Bacon	.006	.030	.108	.096

* Massachusetts General Hospital.

TABLE V

COMPARISON OF ACID BASE VALUES CALCULATED FROM DIFFERENT DATA

	Sherman, 1911		Sherman, 1927		Ca and P from M.G.H. with Sherman, 1927	
	Base cc. N	Acid cc. N	Base cc. N	Acid cc. N	Base cc. N	Acid cc. N
Bread	—	—	—	6.5	—	6.6
Apple	3.8	—	3.5	—	3.3	—
Banana	5.6	—	8.3	—	7.5	—
Steak	—	13.9, 10.1, 12.0, 13.7 (Av. 12.8)	—	16.5	—	13.0
Chicken	—	17.0	—	—	—	10.3 (Sherman, 1911)

weights of protein, the calculated acidity for raw bacon would be nearly twice that for cooked. The problem is complicated not only by the difference in analytical values obtained from different sources for the same food, but also by the difference in salt content of various brands of bacon. Actually, the raw bacon obtained in the Massachusetts General Hospital showed on analysis 43 cc. of normal chloride solution per 100 grams.

The difficulty could be simplified in large measure if patients would eat all the bacon fat eliminated during cooking. In practice, however, it is not easy to persuade patients to do this. Bacon, therefore, is probably best

Table VI
Estimated Excess Acid in Bacon, Raw and Cooked
(Per 100 grams)

	Ca		Mg		K		Na		P		Cl		S	
Total, gm.	0.006	*0.030*	0.012	*.023*	0.169	*0.336*	0.042	*2.7*	0.108	*0.096*	0.038	*4.1*	0.115	*0.229*
Total, cc. N	0.3	*1.5*	1.0	*1.9*	4.3	*8.6*	1.8	*116.5*	7.0	*6.2*	1.1	*116.5*	7.2	*14.3*

Total, cc. N acid	15.3	*137.0*
" " " base	7.4	*128.5*
" " " excess acid	7.9	*8.5*

Note: Figures in italics are for cooked bacon. Other figures represent raw bacon according to Sherman, 1927.

avoided even though it is an excellent constituent for a high-protein, high-caloric, low-calcium diet.

In Table VII is shown a neutral diet. This is more basic than originally intended, for the original estimate based on Sherman's 1911 figures with certain amplifications from this laboratory was 0.1 cc. N acid.

The synthesis of a low calcium diet high in potential acidity is accomplished largely by feeding a maximum of protein, together with cereal products (such as rice and bread); eliminating vegetables (such as potato) or fruits which are potentially alkaline. That the precise excess acid value of such a diet is difficult to determine can be seen from the variation in estimated acidity for steak and chicken already commented upon.

In Table VIII is shown the variation in a very acid diet calculated from two sets of data. This diet constitutes as acid a diet as could be arranged without recourse to foods (like cranberries) yielding a high organic-acid excretion. The variation is approximately 20.0 cc. of N acid per day, which is almost altogether due to the phosphate analyses. It is interesting to note that our original estimate was 72.3 cc. N acid.

In Table IX another similar high acid diet is given, formerly presumed to yield 68.9 cc. N excess acid on the basis of Sherman's 1911 figures with our Ca and P analyses. Calculated from Sherman's 1927 figures plus Massachusetts General Hospital Ca and P this yielded 65.2 cc.

TABLE VII
NEUTRAL DIET

	Gm.	Ca		Mg	K	Na		P		Cl		S
Bread	150	0.040	*0.017*	0.034	0.162	0.591	*0.510*	0.139	*0.122*	0.911	*0.786*	0.157
Butter fat	40											
Bacon*	30	.003	*.009*	.007	.101	.025	*.804*	.064	*.028*	.022	*1.239*	.069
Apple	200	.014	*.020*	.016	.254	.022	.022	.024	*.036*	.010	.010	.012
Sugar	60											
Steak	100	.012	*.008*	.025	.360	.089	.089	.226	*.172*	.080	.080	.244
Potato	250	.035	*.027*	.070	1.072	.053	.053	.145	*.117*	.095	.095	.075
Corn	100	.006	*.005*	.033	.113	.040	.040	.103	*.061*	.014	.014	.046
Banana	100	.009	*.007*	.028	.401	.034	.034	.031	*.042*	.125	.125	.010
Honey	30	.001	.001	.005	.115			.005	.005	.008	.008	
Chicken**	45	.005	*.007*	.016	.209	.042	.042	.115	*.073*	.027	.027	.131
Tomato	125	.014	*.008*	.012	.343	.012	.012	.032	*.068*	.043	.043	.017
Ginger ale	400	.002	.002					.004	.004			
Total, gm.		.141	*.111*	.246	3.130	.918	*1.606*	.888	*.728*	1.335	*2.427*	.761
Total, cc. N		7.00	*5.50*	20.2	80.0	39.9	*69.9*	57.2	*46.9*	37.6	*68.4*	47.4

Total, cc. N base	147.1	*175.6*
" " " acid	142.2	*162.7*
" " " excess base	4.9	*12.9*

Note: Supplementary analyses from M.G.H. Ca & P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

Table VIII
High Acid Diet

	Gm	Ca		Mg	K	Na		P		Cl		S
Bread	300	.081	*.033*	.069	.324	1.182	*1.020*	.279	*.243*	1.821	*1.572*	.315
Bacon*	45	.005	*.013*	.010	.152	.037	*1.206*	.097	*.043*	.034	*1.858*	.103
Steak	200	.024	*.016*	.050	.720	.178	.178	.458	*.344*	.160	.160	.488
Corn	200	.012	*.010*	.066	.226	.080	.080	.206	*.122*	.028	.028	.092
Rice (Cooked Wt.)	100	.003	*.006*	.012	.027	.009	.009	.036	*.075*	.020	.020	.045
Chicken** (dark meat)	60	.006	*.012*	.022	.279	.057	.057	.154	*.094*	.036	.036	.172
Macaroni (raw wt.)	30	.006	*.005*	.011	.039	.002	.002	.043	*.039*	.021	.021	.051
Sugar	30											
Butter fat	40											
Ginger ale	400	.002	.002					.004	.004			
Total, gm.		.139	*.097*	.240	1.767	1.545	*2.552*	1.277	*.964*	2.120	*3.695*	1.266
Total, cc. N		7.0	*4.8*	19.7	45.2	67.2	*111.0*	82.2	*62.1*	59.8	*104.2*	79.1

Total, cc. N acid	221.1	*245.4*
" " " base	139.1	*180.7*
" " " excess acid	82.0	*64.7*

Note: Supplementary analyses from M.G.H. Ca and P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

TABLE IX
HIGH ACID DIET

	Gm.	Ca		Mg	K	Na		P		Cl		S
Bread	300	0.081	*0.033*	0.069	0.324	1.182	*1.020*	0.279	*.243*	1.821	*1.572*	.315
Bacon*	45	.005	*.013*	.010	.152	.038	*1.206*	.096	*.043*	.034	*1.858*	.103
Steak	200	.024	*.016*	.050	.720	.178	.178	.458	*.344*	.160	.160	.488
Rice	100	.003	*.006*	.012	.027	.009	.009	.036	*.075*	.020	.020	.045
Chicken**	60	.006	*.012*	.022	.278	.057	.057	.154	*.094*	.036	.036	.172
Macaroni	30	.006	*.005*	.011	.039	.002	.002	.043	*.039*	.021	.021	.051
Sugar	70											
Butter fat	30											
Ginger ale	400	.002	.002					.004	.004			
Fudge (low Ca)	20			.002	.004			.003	.003			
Total, gm.		.127	*.087*	.176	1.544	1.466	*2.472*	1.073	*.845*	2.092	*3.667*	1.174
Total, cc. N		6.4	*4.4*	14.4	39.4	63.7	*107.6*	62.0	*54.4*	58.9	*103.4*	73.2

Total, cc. N acid	194.1	*231.0*
" " " base	123.9	*165.8*
" " " excess acid	70.2	*65.2*

Note: Supplementary analyses from M.G.H. Ca & P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

of N acid. Using Sherman's 1927 figures alone the result would be 70.2 cc. N acid.

Inasmuch as it was desirable for certain purposes to keep the calcium content of our diets at a minimal level of about 100 mg. of calcium per day, it was not found feasible to construct adequate diets more alkaline than 40 cc. of N base per day without exceeding this limit. With a high calcium content it is not hard to exceed 150 cc. of N base daily on an adequate diet (with sufficient protein).[4] In our experiments, however, it was necessary to rely upon additions of sodium bicarbonate and sodium citrate to produce extreme alkalinity.

The following table (X) illustrates the most alkaline mixed diet, adequate in protein but low in calcium, that we could devise for a patient. The excess base amounts to only 30 cc. N alkali.

TABLE X
LOW CALCIUM MAXIMUM BASIC DIET

	Grams Daily	Acid cc. N	Base cc. N	Protein Grams	Calcium Grams
Apple	150		5.64	.45	.015
Crackers	60	4.68		5.88	.013
Bacon	30	2.52		6.9	.009
Potato	500		31.70	9.5	.055
Banana	250		13.9	2.75	.017
Steak	100	12.8		21.3	.008
		20.0	51.2	46.8	.117

Total cc. N excess base—31.2

DISCUSSION

It is evident that an error of 10 cc. or more of N acid may commonly be encountered in calculating the potential acidity of a daily diet according to the data employed. The production of organic acids is another uncertain factor in computing an acid base balance. In view of the uncertainty, however, which attends bowel activity (absorption, intestinal rate, excretion) and which is in large measure uncontrolled, such calculations afford an adequate basis at least for interpreting changes in excretion associated with quantitated variations from a constantly continued "control" diet.

In any case, if the actual weighed dietary records be preserved, they may always be recalculated in the light of further analytical knowledge.

[4] See Sansum, W. D., Blatherwick, N. R., and Smith, F. H., *Journal of the American Medical Association*, LXXXI, 883, 1923. These authors present an alkaline diet qualitatively, but say nothing as to its total excess base content.

The difficulties of calculating exactly acid base balance can be simplified greatly if the diet consist of only a very few simple ingredients. This procedure, however, is not practicable with patients over a long period of study. In this series of studies patients were given a simple mixed diet which was maintained uniform, as nearly as possible, in order to determine the effect of quantitative variations in simple foods.

Analysis of Specimen Diets

In view of the difficulties already described in calculating the potential acidity of diets, it seemed desirable to ascertain this value directly for comparison with the calculated values. It was at first intended that this should be done by combustion of a sample in a calorimetric bomb; but it

Table XI

Comparison Between Analyses and Estimated Content of Daily Diet

	Diet I Neutral		Diet II Neutral		Diet III Acid		Diet IV Acid		Diet V Basic	
	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N
Phosphorus	49.0	41.9	57.2	41.4	82.0	56.4	69.0	55.5	47.1	35.2
Chlorine	70.2	68.0	72.0	75.9	111.0	116.2	111.0	92.2	66.9	63.4
Sulfur	39.0	27.9	47.2	46.7	79.0	71.6	81.4	53.8	35.8	34.5
Total acid	158.2	137.8	176.4	164.0	272.0	244.2	261.4	201.5	149.8	133.1
Total base	171.2	124.8	180.6	159.5	189.8	177.4	174.6	147.5	190.3	159.0
Net acidity	−13.0	+13.0	−4.2	+4.5	+82.2	+66.8	+86.8	+54.0	−40.5	−25.8
Difference	26.0		8.7		15.4		32.8		14.7	

was pointed out to us by Doctors Francis G. Benedict and H. C. Sherman that the inconstant formation of nitric acid under such conditions would lead to gross inaccuracy until a suitable technique might be evolved. For the time being, therefore, it was necessary to be content with individual analyses of the total base and the acidic elements of one day's diet which, summated, could be compared with the calculated values.[5] The results ob-

[5] The entire diet for one day was finely ground, mixed thoroughly, dried, and powdered. The analyses were made both on samples of powdered dry diet and on nitric acid extracts of diets. Fiske's methods for total base, phosphate, and total sulfur were employed, and Van Slyke's for chloride. We are indebted to Miss Dorothy M. Tibbetts and Miss Phoebe E. Drury for these results.

tained from five typical diets are recorded in Table XI in which the theoretical estimates were obtained from Sherman (1927) supplemented by our analysis of cooked bacon.

It is apparent that the potential acidity as calculated is not very accurate and that deviations from neutrality were actually much less marked than anticipated.

It has become progressively more obvious during these studies upon inorganic salts that variations should only be made very slowly as not infrequently a week elapses before the patient has attained a steady state after a variation in diet. Many of the results in the literature are, therefore, of little value because they represent only transitional states due to the brevity of observation.

The complexity of metabolic processes occurring within the body, moreover, is such that marked changes in the diet may result in only moderate variation in excretion levels (7).

The urinary response to such diet is indicated by a few instances in Table XII, from which it will be observed that the response to change in diet is significant only in a qualitative sense. The first of these patients had tetany (due to poor fat assimilation), and the last two were essentially normal.

TABLE XII

POTENTIAL ACIDITY OF DIET *vs.* ACIDITY OF DAILY URINE (Average)

	Estimated acidity of diet cc. N Calc'd	Found	Titratable acidity $-CO_2$ of urine cc. N	Urinary ammonia cc. N	Total acid excretion cc. N
DeBa. neutral	−13.0	+13.0	−5.5	60.6	55.1
Da. neutral	−4.2	+4.5	16.7	24.1	40.8
Da. high acid	+82.2	+66.8	18.2	45.8	64.0
An. neutral	+1.0		−20.9	38.9	18.0
An. high acid	+86.8	+54.0	−19.5	104.9	85.4

There is obviously much to be desired in improvement of the calculation of potential acidity in diets. For careful metabolic work it is apparently imperative either to analyze each diet as fed, or to feed extremely simple

foodstuffs. It is at least essential to employ a constant basal diet to which potentially acidic or basic additions may be made.

Summary

1. The calculation of the potential acidity or alkalinity of diets is discussed.

2. Results so estimated with the help of standard nutrition tables are compared with actual analyses and with the urinary acid excretion on feeding such diets.

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SOME FACTORS AFFECTING ACCURACY IN THE COLLECTION OF DATA ON THE GROWTH IN WEIGHT OF SCHOOL CHILDREN*

By

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A STUDY on the growth in height and weight of Texas school children is being conducted by the Division of Rural Home Research of the Texas Agricultural Experiment Station in cooperation with the public schools of San Antonio. The elementary supervisors selected three schools to participate in this project. Each school provides a representative population of from 500 to 600 of one of three racial groups, white, Mexican, and negro.

The object of the project is to determine whether Texas school children exhibit seasonal variations in their growth curves, and whether the three racial groups show characteristic or significant differences in their physical development. In order that the study may contribute to our more exact knowledge concerning physical growth processes, special attention has been given both in the preliminary plans and subsequent procedure, not only to employment of suitable equipment and good technique, but also to the collection of data under as nearly uniform conditions as possible.

This study belongs to the individualized type, inasmuch as the growth of individual children is being followed over a period of time. Individualized studies stand in contrast to the older generalized type, in which a single measurement is taken on each member of a large group. Averages are then derived from these single measurements.

The collection of data for this study has already been in progress through one and one-half school years. Some facts obtained well justify the provision for a known weight of clothing to be worn while weight is being taken, weighing at approximately the same hour of day each month, and requiring each child to empty the urinary bladder immediately before weighing. These three procedures were adopted as routine at the beginning of this study. Their importance is emphasized by an analysis of the data pertaining to them. It seems logical to present these data in such manner as will

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call attention to the performance of children as individuals. Averages that could be derived from the figures seem of less interest than the relation between actual monthly changes in nude body weight of individuals and each of the three factors to be considered in this report.

Clothing weights compared with body weight changes. Continental scales of platform type, calibrated in ounces, are used. Uniformity of clothing worn at the times of weighing is insured by the use of garments of known weight. This garment weight is checked from time to time. Thus the nude body weight of each child is obtained. Indoor clothing exclusive of shoes, wraps, pocket contents, jewelry, beads, and girls' heavy belts has been weighed at five different monthly weighing periods. Three seasons are represented: the fall weights were taken in October; the two winter, in January and February; and the two spring, in March and April.

With the assumption that a group of 100 children would constitute a fair sample, a comparison has been made between the clothing weights and the monthly weight changes of 99 white children, 51 boys and 48 girls. These children were in the fourth and fifth grades, and for the most part were 10 and 11 years old.

Four-fifths or more of the maximum monthly body weight changes for both boys and girls occurred in either a winter or a spring month, with an approximately even division between the two seasons. With two exceptions, the maximum changes were gains. More than four-fifths of the minimum body weight changes for both sexes also occurred in either a winter or a spring month. For the boys nearly twice as many minimum changes came in a spring month as in the winter; for the girls about one-third more, in the spring. Among the minimum changes the gains and losses are about equally divided, and with few exceptions are 4 oz. or less.

Maximum clothing weights ranging from 21 to 40 oz. for boys and from 9 to 31 oz. for girls, fell in the winter months in four-fifths or more of the cases. Roughly three-fifths of the minimum clothing weights varying between 15 and 30 oz. for boys came in the spring; nearly two-fifths in the fall. For the girls the minimum clothing weights, varying from 6 to 13 oz., were equally divided between spring and fall. Fewer than 10 minimum clothing weights for either sex were found in a winter month.

With the drop from maximum to minimum clothing weight in the spring, which occurred three times out of five among 51 boys and half the time for 48 girls, any gain in body weight would seem to be less than it really is, had the child been weighed in his indoor clothing. If the body weight change is a loss, then the loss is augmented by the drop from maxi-

mum to minimum clothing weight. On the other hand, a change from minimum to maximum clothing weight might conceal the fact that the child has actually lost weight, or else materially minimize the loss. The case of G. R. in Table I illustrates this possibility. His maximum clothing

TABLE I

CLOTHING WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Boys	Monthly changes in body weight			Clothing weight			Clothing weight difference equals or exceeds monthly wt. changes
	Max.	Min.	Changes greater than clothing wt. difference*	Max.	Min.	Diff.	
	lb–oz.	oz.	oz.	oz.	oz.	oz.	Times
N. C.	+1–15	−1	29, 31	29	17	12	8 out of 10
H. R.	+3–7	+1	18	35	20	15	6 out of 9
H. J.	+3–6	−2	20	32	22	10	4 out of 6
D. A.	+2–1	+1	17	31	18	13	7 out of 10
W. R.	+1–10	−3	17, 20	31	16	15	7 out of 10
F. G.	+3–13	−1	23	39	22	17	7 out of 10
H. H.	+2–13	+1	11, 17	24	17	7	6 out of 10
C. J.	+2–3	−2	12, 14, 17	31	22	9	6 out of 10
H. C.	+2–10	+1	15, 17, 19	34	21	13	6 out of 10
P. J.	+4–4	+2	12, 12, 15	36	27	9	5 out of 10
T. O.	−1–10	+3	12, 14, 18, 21	28	18	10	5 out of 10
J. J.	+2–12	+1	18, 19, 20	30	15	15	5 out of 10
G. C.	+1–15	±3	16	26	19	7	4 out of 10
C. E.	+3–1	+4	12, 15, 18	38	28	10	4 out of 10
F. J.	+2–10	−8	12, 13, 16	31	20	11	4 out of 10
E. R.	+3–1	±0	14, 15, 17, 20	34	23	11	4 out of 10
E. F.	+3–12	−6	17	29	20	9	3 out of 8
B. R.	+2–4	+4	20, 21, 21	35	17	18	3 out of 8
W. B.	+3–12	−1	18, 22	28	17	11	3 out of 10
S. R.	+1–3	±0	11, 19, 19	31	22	9	3 out of 6
N. N.	+2–4	+1	14, 15	30	22	8	2 out of 6
S. T.	+1–1	±2	17	35	29	6	2 out of 3
McM. J.	+1–1	−1	17	33	23	10	2 out of 3
G. R.	−1–3	+5	19	27	17	10	2 out of 3
D. G.	+0–14	+5	14	33	20	13	2 out of 3
B. W.	+1–15	−5	31	40	25	15	2 out of 3

* Those which were 3 or more times the difference are omitted.

weight is 27 oz.; minimum clothing weight, 17 oz.; loss in body weight from preceeding month, 19 oz. Had his body weight been increased by the 10

oz. difference in clothing weights he would appear to have lost but 9 oz. rather than 1 lb. 3 oz.

The extreme variations in clothing weights are of a distinctly higher order than the minimum nude body weight changes for most of the 99 children. By comparing each child's difference between maximum and minimum clothing weight with *all* his recorded monthly body weight changes, it was found that for approximately half the children, the difference between maximum and minimum clothing weights equalled or exceeded his monthly body weight changes, 30 per cent or more of the time. On the basis of this finding, the data for 26 of the 51 boys and 20 of the 48 girls are regarded as significant. These data are recorded in Tables I and II. Eighteen of the 26 boys or 69 per cent, and 8 of the 20 girls or 40 per cent,

TABLE II

CLOTHING WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Girls	Monthly changes in body weight			Clothing weight			Clothing weight difference equals or exceeds monthly wt. changes
	Max.	Min.	Changes greater than clothing wt. difference*	Max.	Min.	Diff.	
	lb.–oz.	oz.	oz.	oz.	oz.	oz.	Times
W. M.	+2–4	±0	12, 18	17	7	10	6 out of 9
W. B.	+1–2	±1	7	11	8	3	6 out of 10
G. A.	+2–8	+4	24, 26, 32, 40	31	11	20	6 out of 10
S. F.	+2–10	±0	10, 17	18	9	9	5 out of 10
S. M.	+2–9	±0	11, 12, 16	16	6	10	5 out of 10
C. H.	+1–3	+1	15, 15, 19, 19	18	8	10	5 out of 10
N. L.	+2–7	+3	14, 15	15	8	7	4 out of 10
J. V.	+2–4	±4	10, 15	15	7	8	4 out of 10
W. D.	+3–11	±0	13	16	7	9	4 out of 10
M. W.	+3–10	+1	22	21	7	14	4 out of 10
S. L.	+2–6	−1	16, 18, 20	20	6	14	3 out of 8
J. R.	+3–3	+11	16, 20, 21	23	8	15	3 out of 8
B. M.	+3–0	−1	14, 17, 20	17	8	9	3 out of 10
B. K.	+2–15	+4	28	20	8	12	3 out of 10
T. S.	+5–5	+1	19, 23, 26	23	7	16	3 out of 10
Y. D.	+2–10	−6	13	14	8	6	1 out of 3
S. P.	+2–7	−2	0	14	6	8	1 out of 3
R. M.	+3–2	−2	0	17	8	9	2 out of 6
W. B.	+1–2	+4	13, 17	18	8	10	2 out of 4
E. M.	+1–0	−1	16	20	5	15	3 out of 4

* Those changes which were 3 or more times the difference are omitted.

exhibit still greater significance, since one-half or more of the total number of nude body changes recorded for each child are equalled or exceeded by his clothing weight difference.

Even the values of the monthly body weight changes which fall outside the limits of the definition of significance here used, point further to the necessity of always weighing with a known amount of clothing those children whose growth is being followed month by month. Among the 26 boys there are 53 monthly weight changes which exceed clothing weight differences, but these monthly body weight changes for any child are never so much as three times his clothing weight difference. In 44 of the 53 instances the monthly body weight changes exceed the clothing weight difference by amounts varying from 1 or 2 oz. up to twice the clothing weight difference. The situation is similar for the girls. Eighteen girls have 40 monthly body weight changes which in each individual case are greater than the clothing weight difference for that girl. Out of the total 40, 35 changes exceed the clothing weight difference by 1 or 2 oz. up to twice the clothing weight difference.

This interpretation of the above data for 46 of 99 children indicates that the truth about monthly body weight changes cannot be known if the child is weighed in his varying amounts of indoor clothing.

Time-of-day weight differences compared with body weight changes. The normal fluctuation in body weight from hour to hour on any day for any individual, although recognized, is not often taken into account when changes in weight are being followed. The authors of this report, by weighing themselves at hourly intervals, each found extreme variations of 2-1/2 to 3 lbs. per day. Dr. T. Wingate Todd[1] notes that his "own weight with all precautions taken varies 5 lbs. in a day, and those of my children, 3 lbs."

In this project the weighing time is kept as nearly constant as possible. Within each half day, the variation of weighing time in successive months is seldom as much as two hours, more commonly less than one hour. Thus the time relationship of weighing hour to food intake and regularly occurring defecation is more uniformly maintained. Monthly weight changes are derived by subtracting the weights of two consecutive months taken at approximately the same hour of day, and with the usual attention to other factors affecting accuracy. Such weight changes are designated as *actual* in this report. The data for 22 children who by mistake were weighed in the morning instead of the afternoon, and the weighing repeated in the after-

[1] Personal communication.

noon of the same day, have been studied with respect to the bearing this time-of-day factor has upon accuracy in following the child's monthly change in weight. Sixteen of the children were white boys from 7 to 8 years old, the other 6, adolescent negro girls. For each of these children an *apparent* monthly weight change is the difference between his weight taken at a time other than his usual hour,and his weight at the usual hour in the preceding month, other conditions being the same.

Among 11 of the 22 children the difference between apparent and actual change was insignificant, one difference being 3 oz.; five, 2 oz.; three, 1oz.; and two, 0. The data in detail for the other half of the children whose apparent body weight change differs from the actual by 4 oz. or more are included in Table III.

TABLE III

TIME-OF-DAY WEIGHT DIFFERENCES COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

White Boys	Body weight: Jan.	Body weight: Change in wt. Jan.–Feb.: Actual	Appar-ent	Diff.	Monthly changes in body weight: Max.	Min.	Actual changes greater than the difference* between apparent and actual change	Difference between apparent and actual change equals or exceeds monthly wt. changes
	lb.–oz.	lb.–oz.	lb.–oz.	lb.–oz.	lb.–oz.	oz.	oz.	Times
E. G.	46–4	+3–3	+2–5	−0–14	+3–3	−1	16, 18, 24	6 out of 10
S. J.	47–12	+1–4	+0–9	−0–11	+1–6	−1	18, 19, 20, 22	6 out of 10
R. A.	74–14	+2–14	+2–2	−0–12	+2–14	+5	19	1 out of 3
S. J.	47–3	+0–3	+0–10	+0–7	+1–7	+2	8, 11, 13, 15	4 out of 10
T. W.	45–13	+1–9	+1–5	−0–4	+1–9	+3	5, 6, 7, 7, 11, 11	1 out of 10
P. P.	59–5	+1–12	+1–6	−0–6	+1–12	+5	9, 10, 12	1 out of 10
Negro Girls								
H. C.	102–3	+1–4	+2–13	+1–9	+1–5	−3	0	10 out of 10
G. S.	121–8	−1–0	+1–14	+2–14	+2–10	+6	0	8 out of 10
R. V.	88–11	+3–4	+4–11	+1–7	+3–4	+6	0	5 out of 6
R. V.	91–12	+0–8	+3–9	+3–1	+3–2	+2	0	9 out of 10
M. P.	100–8	+0–14	+4–0	+3–2	+4–2	+2	52, 57	7 out of 10

* Those changes which were 3 or more times the difference are not shown.

The number of times the difference between apparent and actual change on the day of double weighing,equalled or exceeded the observed monthly

changes for each child is high, except for two boys. For one of these boys (T. W.) the body weight changes which were greater than the differences between apparent and actual change, range from 5 to 11 oz. in six changes out of ten; for the boy (P. P.) three of ten changes have values of 9 to12 oz. Since for these two children the differences between apparent and actual change are 4 and 6 oz. respectively, all of their monthly weight changes considered together would seem sufficient reason for retaining them in the significant group. The actual nude body weight changes for the boys range from plus 3 oz. to plus 3 lbs. 3 oz., and are greater than the apparent change in each case by differences of 4 to 14 oz. The total number of monthly body weight changes which are equalled or exceeded by the difference between apparent and actual body weight change, expressed in percentage, are respectively 10, 10, 30, 40, 60, and 60.

The apparent changes credit each of the negro girls with a gain, whereas one girl actually lost 1 lb., the others gained from 8 oz. to 3 lbs. 4 oz. The errors for the monthly weight changes range from plus 1 lb. 7 oz. to plus 3 lb. 2 oz. Here, the total number of monthly body weight changes which are equalled or exceeded by the difference between apparent and actual body weight change, expressed in percentage, are respectively 70, 80, 83, 90, and 100.

These data on this small group of children add to the evidence that monthly weighing should be done at some fixed hour in the day.

Urine weight compared with body weight changes. The failure of a few children to comply with the routine requirement to empty the urinary bladder immediately before weighing, necessitated reweighing them. This necessity suggested that amounts of urine commonly eliminated at one voiding be determined. Accordingly, 38 boys and 20 girls were weighed just before and again directly after voiding. This determination of urine weight was made immediately before each child's body weight was taken in April of 1930. The urine eliminated by half of the children weighed 3 oz. or less. Table IV contains the data for the other half of the group, 19 boys and 10 girls, who voided 4 oz. or more of urine, and for whom the urine weight has been compared with monthly nude body weight changes.

There is no significant difference as between the sex groups in the weight of urine passed by the individuals; the maximum figure for the boys is 16 oz., for the girls 13 oz. Neither is there a significant difference in absolute amounts of urine eliminated at one voiding by children and adults. In 11 determinations for three adult women, three weights were 8, 11, and 13 oz. respectively; eight weights fell upon 3, 4, 5, 6, and 7 oz. A consideration of

the data in Table IV suggests that a factor which may influence the body weight by an amount of 4 oz. or more must not be disregarded. Minimum

TABLE IV

URINE WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Boys	Body wt.		Wt. of urine voided	Monthly changes in body wt.			Urine wt. equals or exceeds monthly changes in body wt.
	March	Change in wt., Mar.–April		Max.	Min.	Body wt. changes greater than urine wt.*	
	lb.–oz.	lb.–oz.	oz.	lb.–oz.	oz.	oz.	Times
S. H.	49–5	−0–3	7	+1–14	2		5 out of 6
A. M.	113–1	−0–4	16	+4–2	+2	27	4 out of 7
S. L.	59–10	+1–7	4	+1–7	±2	6, 8	5 out of 10
C. W.	48–3	+0–3	8	+2–2	±3	10, 14	5 out of 10
B. L.	51–6	+1–12	8	+1–12	−2	10, 19	4 out of 8
McC. J.	70–11	+1–5	4	+2–3	±0	5, 8, 8	3 out of 8
P. G.	47–15	−0–10	6	+1–0	+4	8, 9, 10, 12	3 out of 8
P. C.	66–1	+0–9	7	+3–2	+4	9, 14	3 out of 8
P. N.	73–15	+1–3	5	+2–1	±0	8, 13, 14	4 out of 10
B. W.	91–15	+1–3	10	+2–4	+3	14, 18, 19	3 out of 10
A. B.	80–0	+3–11	7	+3–11	+3	13, 14	2 out of 10
N. C.	60–11	+1–12	4	+3–3	−4	6, 7, 9, 10	1 out of 10
R. J.	82–9	+0–9	4	+3–6	−4	6, 8	1 out of 10
W. L.	abs.		4	+4–1	−3	8, 11	1 out of 8
S. J.	76–15	+0–3	6	+3–2	+3	8	2 out of 7
R. G.	80–11	+0–10	5	+1–12	+9	9, 9, 10, 11, 14	0 out of 8
G. C.	57–12	−0–5	9	−1–7	+10	10, 11, 11, 15, 15	0 out of 7
E. H.	38–0	+1–9	4	+1–9	−4	0	1 out of 2
W. H.	63–11	+0–14	13			14	0 out of 1
Girls							
B. V.	48–10	−0–3	13	+1–2	±3	14, 18	8 out of 10
H. M.	65–3	±0–0	10	+1–6	±0	11	3 out of 5
N. A.	75–14	+0–6	9	+4–3	−4	0	4 out of 6
S. F.	78–0	+0–5	9	+2–10	±0	10, 17	5 out of 10
N. L.	60–4	+0–3	7	+2–7	+3	14, 15	4 out of 10
S. C.	46–15	+1–5	4	+1–5	±0	5, 8, 11	3 out of 7
T. R.	62–6	+1–5	5	+1–8	−4	6, 8, 13	2 out of 10
N. G.	50–5	+0–9	4	+1–11	+9	9, 11	1 out of 5
H. A.	90–13	−0–2	4	+4–0	−2	0	1 out of 5
C. P.	abs.		5	−2–4	+8	8	0 out of 4

* Those changes which were 3 or more times urine weight are omitted.

monthly nude body weight changes are frequently 4 oz. or less, and changes of 5, 6, and 7 oz. are not uncommon. For 11 of the 19 boys and 6 of the 10 girls the urine weight equalled or exceeded the monthly body weight changes in one-third or more of the total number of monthly changes recorded. For six boys, and four girls, the narrower limit of one-half or more of the total number of monthly body weight changes being equalled or exceeded by urine weight, holds true. Each of twenty-five children shares in a distribution of 60 monthly nude body weight changes greater than his urine weight. In 48 of these 60 instances, the monthly body weight changes exceed the urine weight by amounts varying from 1 or 2 oz. up to twice the urine weight. Only in 9 of the 60 instances do the monthly body weight changes approach three times the urine weight.

With respect to changes calculated from the April weighing, several individual cases are of particular interest. W. H., who had only two consecutive monthly weights, with 13 oz. of urine, practically equalled his 14 oz. gain. A. M. who eliminated 16 oz. of urine which exceeded his monthly weight change four times out of seven had two losses of 4 oz. each, one gain of 2 oz. and another of 8 oz. A. M. is one of six children whose April weight record showed a failure to gain. The actual changes of April from March body weight for these six children were respectively ± 0, -3, -3, -4, -5, -10 oz. Had the urine not been eliminated the apparent changes arranged in corresponding order would have been $+10$, $+4$, $+10$, $+12$, $+4$, -4 oz. Obviously, emptying the urinary bladder is an imperative procedure not to be neglected if the facts regarding monthly body weight changes are to be ascertained.

Summary

In following the weight of school children from month to month the data here presented show that accuracy cannot be attained unless, along with attention to the many other influencing factors, the child is weighed, 1.– in a known weight of clothing, 2.– at approximately the same hour of day each month, and 3.– with his urinary bladder empty.

FACTORS WHICH DETERMINE RENAL WEIGHT*

IX. ENDOGENOUS PROTEIN METABOLISM

By

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A CONSTANT relationship between the weight of the kidneys and the protein intake has been demonstrated (MacKay, MacKay and Addis, 1928a; MacKay and MacKay, 1930) in male albino rats. This relationship is of such a nature (MacKay, MacKay and Addis, 1928a, b) that an animal receiving no protein should theoretically have about 150 milligrams[1] of renal tissue per 100 square centimeters of body surface. An experiment has been carried out to test this possibility. Ten groups of male rats of varying age were weighed and then placed upon a diet similar to that which they had been receiving except that it was practically devoid of protein. After varying periods of time, depending upon their age—the older they were the longer the interval—these animals were killed and their kidneys weighed and other measurements made in the manner which has been described (MacKay and MacKay, 1927a). Partly because the rats disliked the diet and the caloric intake was not entirely sufficient, and partly because their body weight cannot be maintained on a protein-free diet, these animals lost weight during the period of observation. For this reason the body weight used for the calculation of the body surface[2] as a basis for comparison of the actual kidney weight with the theoretical figure was that of the commencement of feeding. The kidney weight is partly dependent on the amount of protein which a rat eats. Here we want to know

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[1] This figure and kidney weights given elsewhere refer to the average of the two kidneys. The organism actually has twice this amount of renal tissue.

[2] The use of what we term "body surface" ($11.3\times(\text{B.W})^{2/3}$) in this and other papers of this series as a reference standard need not be considered as having a biological significance. The constant relation of kidney weight to the "body surface" (MacKay and MacKay, 1927b) may only be a necessary geometrical relationship, but as such it serves a useful purpose. We suspect however that the relationship may be due to the possibility, that our "body surface" expression is, under normal conditions, probably a fair measure of the maintenance metabolism of the organism as a whole, and that the maintenance metabolism of the renal tissue probably contributes a constant percentage of this total and is dependent on the anatomical mass of kidney.

TABLE I

Group	Age (days)	Body weight (grams)	Body surface (sq. cm.)	Kidney weight (mgms.)	Mgms. renal weight per 100 sq. cm. body surface	Per cent deviation from theory
1	42	83	208	288	139	−7
		70	188	234	125	−17
		62	171	242	142	−5
		66	177	237	134	−11
		63	171	218	128	−15
		62	171	242	142	−5
2	58	118	263	394	150	0
		104	242	366	151	+1
		101	238	322	135	−11
		116	260	387	149	−1
		90	220	318	145	−3
		100	236	315	134	−11
3	80	185	356	500	141	−5
		195	370	582	157	+5
		230	409	666	163	+9
		170	337	544	161	+7
		170	337	545	161	+7
		184	355	561	158	+5
4	80	188	360	575	159	+6
		206	383	612	160	+6
		190	363	600	165	+10
		178	348	517	148	−1
		184	355	565	159	+5
		190	363	592	163	+9
5	122	250	434	600	138	−8
		274	461	668	145	−3
		242	426	654	153	+2
		234	416	572	137	−9
6	122	236	419	466	111	−26
		188	360	452	125	−17
		238	421	622	148	−1
		198	373	600	160	+6
		174	354	568	160	+6
		161	325	530	163	+9
7	184	326	520	906	174	+16
		304	496	728	147	−2
		330	524	804	153	+2
		332	527	828	157	+5

TABLE I (continued)

Group	Age (days)	Body weight (grams)	Body surface (sq. cm.)	Kidney weight (mgms.)	Mgms. renal weight per 100 sq. cm. body surface	Per cent deviation from theory
8	184	244	428	595	139	−7
		280	469	759	161	+7
		250	437	637	146	−2
		260	446	660	148	−1
		310	503	790	157	+5
9	274	360	556	856	154	+3
		320	513	762	148	−1
		274	461	659	143	−5
		310	503	753	150	0
10	274	358	553	880	159	+6
		346	541	871	161	+7
		358	553	853	154	+3
		350	543	773	142	−5

* In place of using the gross body weight for computing this figure an allowance of 5 per cent has been made for intestinal contents so that the "body surface" obtained will be comparable with that on which the theoretical kidney weight is based. The "body surface" is the expression (body weight) $^{\frac{2}{3}}\times 11.3$. In this case the initial body weight was used.

how much kidney weight this rat would have when the factor of protein consumption is removed. This factor is removed and after a time, during which the effect of ingested protein may have a chance to disappear, the kidney weight is compared with that which the rat theoretically would have had at the body surface based on its initial body weight with a zero protein intake. The kidney weight actually found at death must, therefore, be referred to the same body surface for the purpose of comparison. That the arbitrary periods which were chosen for the kidneys to recede in weight, and for their weight to come into equilibrium with the zero protein intake were satisfactory, is suggested by the results.

In Table I are presented the individual observations and their relation to the theoretical figure. According to theory there should be 150 milligrams of renal tissue per 100 square centimeters of body surface if an equilibrium has been established between the weight of the kidneys and the protein intake which in this case is zero. The agreement of the figures actually found with this theoretical figure is very good, particularly when the nature of the data is considered. The greatest deviation of any one age group is

TABLE II

GROUP AVERAGES

Group no.	No. rats	Age		Body weight		Bodysurface	Kidney weight			Kidney wt. mgms. per 100 sq. cm. body surface	Per cent of theory	Per cent deviation
		Initial (days)	Death (days)	Initial (gm.)	Death (gm.)	Initial (sq. cm.)	Left (mgm.)	Right (mgm.)	Average (mgm.)			
1	6	35	42	66	53	179	238	242	240	135	90	−10
2	6	50	58	105	83	244	341	360	350	144	96	− 4
3	6	70	80	189	157	360	567	565	566	156	104	+ 4
4	6	70	80	189	167	360	566	587	576	159	106	+ 6
5	4	110	122	250	218	446	622	618	620	143	95	− 5
6	6	110	122	199	169	377	529	549	539	145	97	− 3
7	4	170	184	323	257	517	788	870	829	157	105	+ 5
8	5	170	184	269	227	457	676	700	688	150	100	0
9	4	260	274	316	286	509	734	766	750	146	97	− 3
10	4	260	274	351	271	546	829	857	843	154	103	+ 3

minus 10 per cent and the average deviation of the entire group of 51 rats from 150 milligrams of renal tissue per 100 square centimeters of body surface is 6.0 per cent. The average of the difference between negative and positive deviations is less than 1 per cent. The average kidney weight in relation to body surface for the whole ten groups is 149.6 as compared with the theoretical 150. The variability of the series, of which the former is an average, is 11.3 per cent which compares very favorably with the 7.0 per cent variability of the relation of renal weight to body surface in male rats of various ages (MacKay and MacKay, 1927b).

The consistent relation which has been demonstrated between the kidney weight and the exogenous protein catabolism as measured by the protein intake (MacKay and MacKay, 1931) led us to examine what relation existed between renal weight and the endogenous protein catabolism under conditions such that the effect of ingested protein was not a factor. Such conditions are those under which the experiment described above was carried out. These rats had been on a diet which is described elsewhere (MacKay and MacKay, 1927b) as the experimental male diet and which contains 18.0 per cent protein. When the observations reported here were commenced, a diet containing the ingredients listed in Table III was given. It is similar to their original diet except that it contains 2 per cent of Harris vitamin B concentrate in place of 9 per cent of yeast, the remaining

TABLE III

EXPERIMENTAL DIET

	Per Cent
Dried cooked cornstarch	68
Lard	15
Cod liver oil	9
Salt mixture (Osborne and Mendel)	4
Yeast extract (Harris Vitamine B concentrated)	2
Agar-agar	2

7 per cent, along with the casein, being replaced with cornstarch. It contained exactly 0.1 per cent nitrogen, which could not be certainly identified as of protein origin and in any case this small amount may be disregarded for the present purpose. The animals were kept in a special urine collection cage very similar to that described by Levine and Smith (1925), except that the collecting portion was entirely of glass. Twenty-four hour group urine collections were made upon all of the ten groups from the time the special diet was commenced until they were killed. At the end of each collection period their bladders were emptied by means of moderate downward pressure over the abdomen while several whiffs of ether were given

(Addis, MacKay and MacKay, 1926). The urine was collected in 10 cc. of 5 per cent sulfuric acid and the cage bottom and collecting funnel washed down at the end of each period. Total nitrogen was determined on the 24-hour specimens by the usual Kjeldahl method.

The nitrogen excretion of each group fell quickly to a minimum which then fluctuated slightly. It seems reasonable to assume that this final minimum nitrogen excretion should be a fair measure of the endogenous protein metabolism. In Table IV are given the group averages. The nitrogen figure is an average of the excretion per rat per day of the last 2 days in

TABLE IV

GROUP AVERAGES

Group No.	No. rats	Age at death (days)	No. days on diet	Body weight (gms.)	Liver weight (gms.)	Heart weight (mgms.)	Kidney weight (aver.) (mgms.)	Minimum N excretion (mgms. per rat per 24 hrs.)
1	6	42	7	53	2.31	239	240	16
2	6	58	8	83	3.37	370	350	15
3	6	80	10	157	5.38	557	566	27
4	6	80	10	167	5.81	581	576	29
5	4	122	12	218	7.14	774	620	43
6	6	122	12	169	5.37	590	539	31
7	4	184	14	257	7.77	840	829	52
8	5	184	14	227	6.79	824	688	38
9	4	274	14	286	8.57	766	750	50
10	4	274	14	271	7.18	847	843	55

groups 1 and 2, the last 3 days in 3 and 4, and the last 4 days of observation in all of the other groups. In Figure 1 these mean figures have been plotted against the mean kidney weight of each group. There is a very definite relation, almost linear, between the two. We believe that this relation is not due to any effect of the endogenous protein metabolism on renal weight but to the relation of both of these figures to the same factor, probably the maintenance metabolism of the organism.

In the experiment which has just been described, the endogenous protein metabolism and the renal weight were varied by the size of the animal which would likewise vary the maintenance metabolism and in similar degree. There is considerable evidence that the endogenous protein metabolism is determined under ordinary conditions by the maintenance metabolism. This suggests that the endogenous protein metabolism: renal weight relationship may be causal in nature. Evidence that this is not the case is presented here. Four groups of male rats 170 days of age were

given the protein-free diet we have described for 4 days. During the next 10 days, two groups continued to receive this food and one in addition daily intraperitoneal injections of thyroxin while the other two groups were given only water. After 6 more days the fourth group received neither food nor water. The nitrogen excretion of all but the first group increased. At

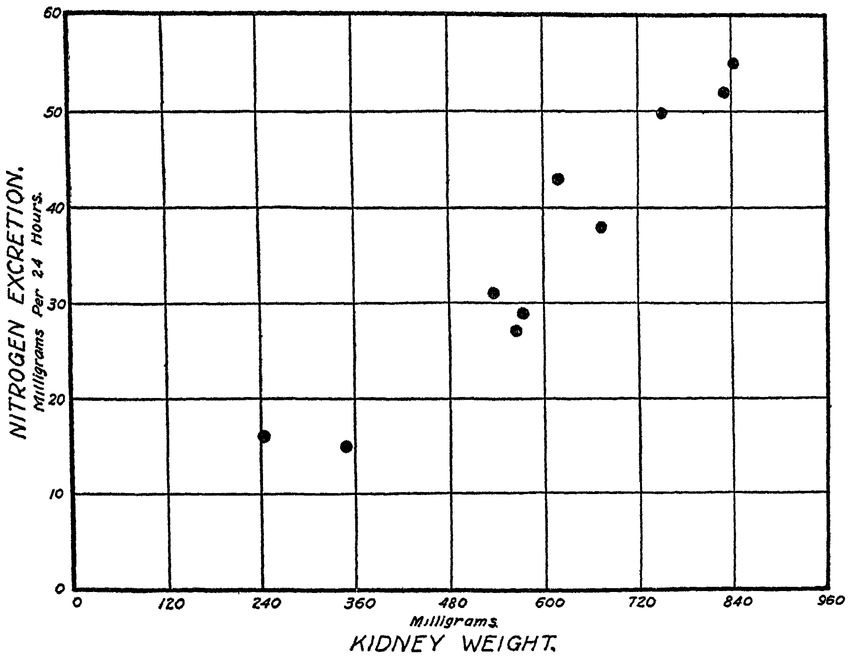

FIGURE 1.

TABLE V
GROUP AVERAGES

Group	No. rats	Body weight: Initial (gm.)	Body weight: Death (gm.)	Initial Body surface (sq. cm.)	Kidney weight: Actual (mgm.)	Kidney weight: Per sq. dcm. (mgm.)	Terminal N. excretion: Per rat per day (mgm.)	Terminal N. excretion: per sq. dcm. per day (mgm.)
Protein-free diet......	5	269	227	457	688	150	38	8
Starvation..	4	273	170	461	603	131	83	18
Dehydration	4	278	155	465	582	125	160	34
Thyroxin...	5	253	152	437	906	208	61	14

the end of 14 days they were killed and the kidneys weighed. The results comprise Table V. Excluding the group which was given thyroxin, the rats with the higher nitrogen excretion did not have larger kidneys. In other words, when the endogenous protein metabolism was increased over that of a protein-free diet by starvation or dehydration, circumstances which would not tend to change the maintenance metabolism or at least not increase it, there was no corresponding increase in the size of the kidneys. Now, on the other hand, when the endogenous protein metabolism was increased in a manner which would also increase the maintenance metabolism of the organism, that is, by the injection of thyroxin, the weight of the kidneys approximately kept pace with the increased nitrogen excretion.

Summary

The kidney weights of male albino rats which have been receiving a diet devoid of protein coincide very closely with the theoretical values which might be expected from the relationship which has been demonstrated between renal weight and the protein intake. The kidney weights of rats receiving a diet containing no protein are almost directly proportional to their endogenous protein metabolism as measured by the urinary nitrogen excretion. Evidence is presented which suggests that this is not a causal relationship but due to their relation to the same factor, perhaps the maintenance metabolism of the organism.

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FACTORS WHICH DETERMINE RENAL WEIGHT*

X. THE EFFECT OF FEEDING DESICCATED THYROID

By

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NUMEROUS observers (R. G. Hoskins, 1910; E. R. Hoskins, 1916; Hering, 1917, 1919; Hewitt, 1920; Cameron and Carmicheal, 1920) have noted the remarkable enlargement of the kidneys which follows thyroid feeding in rats and other animals. An enormous increase in the intake of food (Schater, 1912) and hence protein is a natural accompaniment of thyroid administration and a high protein intake of itself is known (MacKay, MacKay and Addis, 1928) to lead to a marked increase in renal weight. The experiment described here was performed to determine whether the increase in renal weight which results from the ingestion of desiccated thyroid is due to the increased protein intake or to some more fundamental effect of the thyroid principle.

Experiments were carried out with diets containing 0.4, 0.8 and 1.2 per cent desiccated thyroid. The experiment with the 0.4 per cent thyroid diet was alone successful. In the other two all of the animals died of thyroid intoxication before the end of the experiment. In the one successful experiment forty male albino rats exactly 26 days of age were divided into two groups of 20 each. The care of each group was identical. The experimental details and the diet (experimental male) which they received have been described elsewhere (MacKay and MacKay, 1927a). Except for the presence of thyroid in one, the diets of the two groups were the same. The thyroid diet was prepared by intimately mixing 0.4 grams of Armour's powdered desiccated thyroid with 99.6 grams of control food. Having been placed upon the special diets when 26 days old the rats were weighed daily and their food intake determined until they were 70 days of age when they were killed and various anatomical measurements made.

In Figure 1 have been charted the growth curves of the two groups in terms of the mean daily body weight and food intake in relation to the calculated body surface during the period of experiment. Although eating

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far more than the controls, the thyroid group did not increase in weight nearly so rapidly.

The mean results of the anatomical measurements made at death are

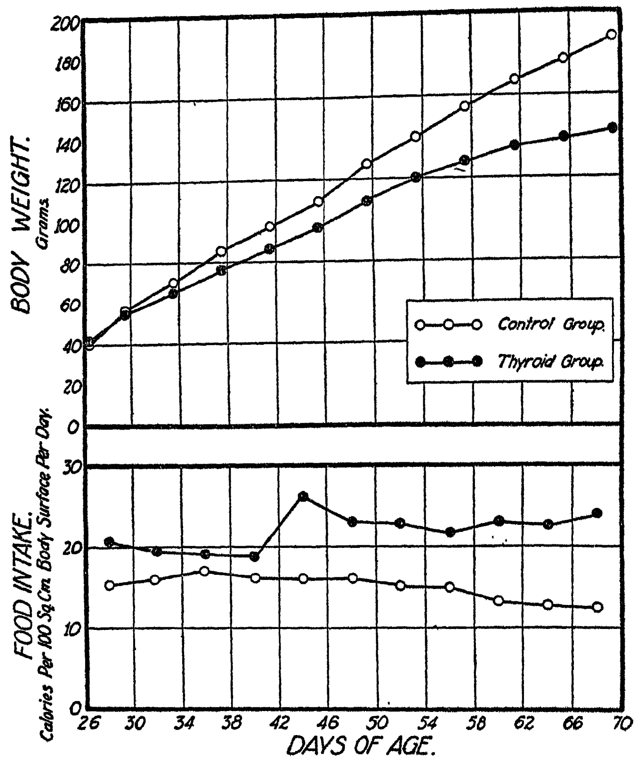

FIGURE 1.

TABLE I
GROUP AVERAGES

	Control group	Thyroid group
No. rats	20	20
Body length, mm.	196	190
Body weight (cor.), gm.	186	138
Body surface, sq. cm.	370	302
Liver weight, gm.	8.25	9.48
Heart weight, mgm.	631	1019
Kidney weight,* mgm.	701	888
Liver weight: Body surface	2.23	3.12
Heart weight: Body surface	171	338
Kidney weight*: Body surface	189	295

* Average of the two kidneys.

given in Table I. They show that thyroid feeding resulted not only in an increase in the weight of the kidneys but also of the liver and heart, an observation which has been frequently recorded before. In Figure 2 the in-

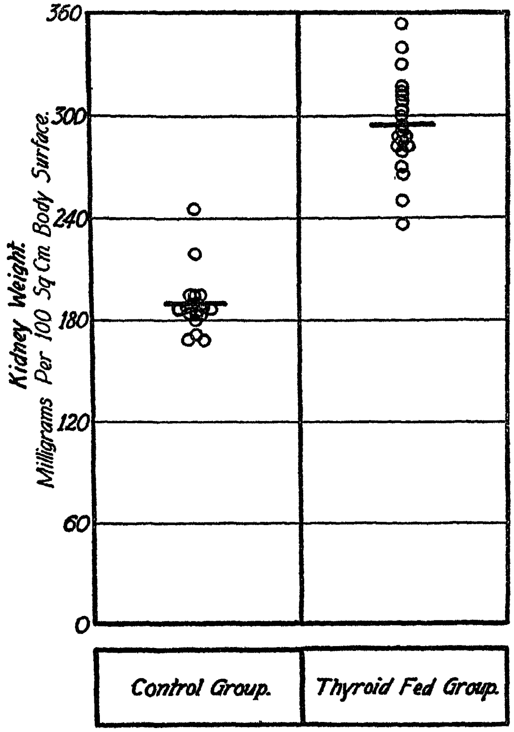

FIGURE 2.

dividual figures for renal weight are presented graphically. In Table II the observed kidney weights of the two groups are compared with their protein intakes and with the calculated weight of renal tissue for these pro-

TABLE II
GROUP AVERAGE

	*Intake—grams per 100 sq. cm. body surface per day		Milligrams kidney per 100 sq. cm. body surface	
	Food	Protein	Observed	Calculated
Control group	2.69	0.484	189	180
Thyroid group	4.93	0.887	295	198

* Average of 10-day period before death.

tein intakes, derived by means of a formula which has been shown (MacKay, MacKay and Addis, 1928) to express the relationship between protein intake and renal weight in albino rats of the age and sex and receiving a diet of the nature of that used here. It is obvious that the size of the kidneys of the thyroid rats cannot be explained solely on the basis of their increased protein intake. Some additional effect of thyroid feeding must contribute to the renal enlargement. The possibility that the increase in the maintenance metabolism of the organism may be responsible for the hypertrophied kidneys at once comes to mind. There are a number of facts which suggest that the maintenance metabolism is an important endogenous factor in determining the size of the kidneys. In normal rats of various ages the renal weight has been found (MacKay and MacKay, 1927b, c) to be directly proportional to the body surface and the latter is directly proportional to the maintenance metabolism of the organism (Lee and Clark, 1929). Then there is the present instance in which the maintenance metabolism is almost certainly greatly increased and so is the renal weight. In contrast to this circumstance are the experiments of Hammett (1927) in which he removed the thyroid gland from young albino rats.[1] From what we know of the results of this procedure in other animals and man, it would seem safe to assume that the maintenance energy demand of these rats was definitely reduced. He found that in relation to the size of the organism as a whole a marked decrease in the weight of the kidneys resulted. It is true that the protein intake of these thyroidless rats would probably be reduced and this of itself might decrease the size of their kidneys. However, recalculating Hammett's figures for his group operated when 30 days old and killed 120 days later for comparison with our data the controls have 191 mgms. and the thyroidectomized group 132 mgms. of kidney tissue per 100 sq. cm. body surface. The latter figure is considerably below that which rats receiving no protein should have theoretically (MacKay, MacKay and Addis, 1928) or are actually found to have (150 mgms.) by experiment (MacKay and Cockrill, 1931). It is planned to examine the relation of renal weight to the maintenance metabolism by direct experiment at some future time.

Summary

The administration of a diet containing 0.4 per cent desiccated thyroid to male albino rats from 26 to 70 days of age was followed by a marked in-

[1] Actually both the parathyroids and thyroids were removed for technical reasons but as Hammet (1926) has pointed out, the response may be interpreted in terms of thyroid deficiency alone.

crease in the weight of the kidneys. This increase in renal weight was greater than could be accounted for by the increase in protein intake incident to the increased food consumption which followed the administration of thyroid. This result is contrasted with the decrease in the weight of the kidneys which results from removal of the thyroid gland. It is suggested that the influence of the thyroid on kidney weight may be through its effect upon the maintenance metabolism of the organism.

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INFLUENCE OF THE STATE OF HYDRATION OF THE BODY ON THE INSENSIBLE LOSS OF WEIGHT IN CHILDREN†

By

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IN THE course of investigations on the total mineral and water exchanges in epileptic children on various regimens, it was observed that the insensible loss of body weight varied to a considerable degree with different levels of water intake and with diets which were isocaloric but of different composition. The insensible weight loss consists almost entirely of water vapor and carbon dioxide and for purposes of practical consideration is equal to the combined output of these substances minus the weight of oxygen simultaneously absorbed. As shown by the calculations of Johnston and Newburg (1), water vapor may constitute 82 per cent of the total insensible loss when the respiratory quotient is 1.00, 104 per cent when it is 0.707, and 92.8 per cent when it is 0.82. Reference to the literature reveals the fact that several investigators have observed a decrease in the cutaneous insensible water loss following restriction of the water intake.

Dennig (2) gives a small number of data from one experiment on an adult, which indicate that the total insensible perspiration is reduced during a period of thirst. His results are inconclusive, however, because his few figures are very variable. Such factors as muscular activity, room temperature and relative humidity are not considered, and the balance used for determining body weight was accurate to 100 grams only. Moog and Nauch (3), using the chamber method of Schwenkenbacher (4) for directly measuring the water evaporated from the skin of the trunk and limbs over short periods of time (7 hours), found in five adult subjects that the insensible water loss from the skin averaged from 7.0 to 40 per cent more when 3000 cc. instead of 1000 cc. of water were drunk. Schlüter (5), using the same method, found that when the fluid intake of two adults was reduced to a low level, the water eliminated through the skin fell from 22.7 to 18.7,

* Read at the annual meeting of the Eastern Society for Pediatric Research at Baltimore, May 3, 1930.

† This investigation was aided by grants from an anonymous donor and from the research fund of the Rockefeller Foundation.

and 18.3 to 15.3 grams per hour respectively. Marfan and Darlencourt (6) observed that infants with dehydration from diarrhea gave off increased proportions of water by way of the lungs. On first thought, this might indicate the possibility that the diminution in insensible water loss from the skin, as observed by Schlüter, is compensatory to an increase in the amount lost by way of the respiratory pathway during dehydration. There is no evidence, however, of a significant increase in the minute respiratory exchange in the type of dehydration resulting from simple water restriction; whereas, this is the usual effect of the acidosis accompanying dehydration from diarrhea. Erismann (7), whose technique was poorly controlled, reported finding an increase in the amount of water evaporated from the skin when the water intake was greatly increased. Peiper (8) found that the insensible cutaneous water was not increased by drinking large amounts of cold water. Laschtschenko (9), who measured the total insensible water output (skin and lungs) after increased water drinking, obtained results similar to those of Peiper.

Special interest in problems pertaining to changes in the insensible "perspiration" or insensible "loss" has been aroused recently by the observation that, under certain conditions, there is a close correlation between the energy metabolism and the net loss in body weight over a given period of time. In 1917 Soderstrom and DuBois (10), using a respiration calorimeter, found, in a fairly varied series of adult human subjects, that approximately 24 per cent of the heat produced in the body was lost by vaporization of water from the skin and lungs, when the environmental temperature ranged between 22 and 24° C. and the relative humidity between 15 and 40 per cent. Benedict and Root (11), using a highly sensitive Sauter balance (sensitive to 0.1 gm.) for determining changes in body weight over short periods of time and so-called "silk scales" (sensitive to 10 grams) for longer periods, demonstrated a definite relationship between the insensible loss and the energy metabolism in adult diabetic and hyperthyroid patients as well as in normal subjects. They supply a table by means of which the 24-hour metabolism may be approximately predicted from the hourly insensible loss measured under standard conditions. Levine and Wilson (12) have made an extensive and well-controlled study of the relationship between basal insensible loss and energy metabolism in infants and children with results comparable to those of Benedict and Root in adults. Johnston and Newburg (1) have extended the method to determine the total heat elimination over longer periods, during which various diets may be used and a limited amount of muscular activity indulged in.

The combined results of these authors have definitely established the practical utility of predicting the metabolism from measurements of the insensible loss. The only standard requirements stated so far are those having reference to the relative humidity and temperature of the environment for the avoidance of sensible sweating or chilling of the skin surfaces.

Because of the increasing importance of measurements of the insensible perspiration, and because of the indefiniteness regarding its status in different states of hydration, we have determined its value under a considerable variety of conditions in conjunction with other studies on the water metabolism in children. The results presented in the present paper are representative of a larger number of data all pointing to similar conclusions.

Plan of Study

The environmental and metabolic conditions, except for the changes induced in the state of hydration, were maintained as nearly constant as possible throughout each experiment.

The subjects, four epileptic girls ranging in age from 11 to 16 years and one boy aged 9 years, were kept in bed under sufficient bed covers to prevent chilling or sensible sweating, so far as possible. The barometric pressure, temperature, and relative humidity in the metabolism room were recorded hourly. The environmental temperature ranged between 22° C and 25° C throughout almost the entire period of observation, only occasionally rising high enough to induce slight moistness in the axilla.

The experimental day was divided into four 6-hour periods, beginning at 6:00 A.M. The method used for estimating the total metabolism was that outlined by Johnston and Newburg (1). At the beginning of each period, the patient's bladder was completely emptied, the weight was then determined on a special scale, sensitive to 5 grams and weighing to 70 kilograms, after which a standard meal of known weight and composition was given. The four meals of the day were identical in every respect and were accurately prepared in an adjoining kitchen from simple substances such as whole milk powder,[1] calcium caseinate,[2] sucrose, eggs, heavy cream, and distilled water. No sodium chloride was added, excepting in one experiment. At the end of the period the patient again voided and was weighed. The difference between the body weight at the beginning of the period plus the weight of the ingesta and the body weight at the end of the period plus the weight of the excreta, gave the total insensible loss for the period. This

[1] Klim.

[2] Casec.

value, divided by the number of hours making up the period, gave the insensible loss per hour, from which energy metabolism was predicted by reference to the tables of Benedict and Root.

Since control periods were run for each diet under normal conditions of hydration, changes in insensible loss due to differences in the respiratory quotient need not be taken into consideration. The diets for each experiment were isocaloric throughout the period of study and were calculated to furnish sufficient energy to maintain body weight under ordinary conditions.

Water balances of the body were calculated according to the method suggested by Newburgh and Johnston (13). "Total water available" (intake) includes drinking water, preformed water of the food, water from oxidation of the metabolic mixture and nonoxidation water freed by the catabolism of body tissues. The "total water lost" (output) includes water of the urine, water of the feces, water lost insensibly by way of the skin and respiratory tract and the water stored with any fat protein or carbohydrate deposited in the body.

In the course of our water balance studies varying degrees of dehydration have been produced in the different subjects by means of rigid water restriction, diuresis, catharsis, low-carbohydrate diets, or variation in the mineral content of the diet. In some instances a combination of two or more of these procedures has been employed. Superhydration has been induced by forced water drinking and frequently repeated administrations of the antidiuretic principle of the hypophysis (pitressin).

Results of Experiments

For the sake of brevity, results of the experiments are presented in a series of graphical charts and one table, which are practically self-explanatory.

The data in Chart 1 show that a significant decrease in the insensible perspiration follows the development of dehydration. The extent of the latter is indicated by the large daily water deficit and by a precipitous fall in body weight during the second half of the experiment. The control period is not entirely satisfactory because of slight sweating on the second and third experimental days. Any error, however, which might result from this during dehydration would naturally be in the direction opposite to that upon which our conclusion is dependent. It is interesting that the skin became extremely dry during the period of dehydration in spite of the fact that the mean room temperature rose on the sixth day to a point one

degree higher than that at which sensible perspiration had occurred in the control period. It is apparent from the results for the first four days that the non-ketogenic, borderline, high-protein diet had only a feeble dehy-

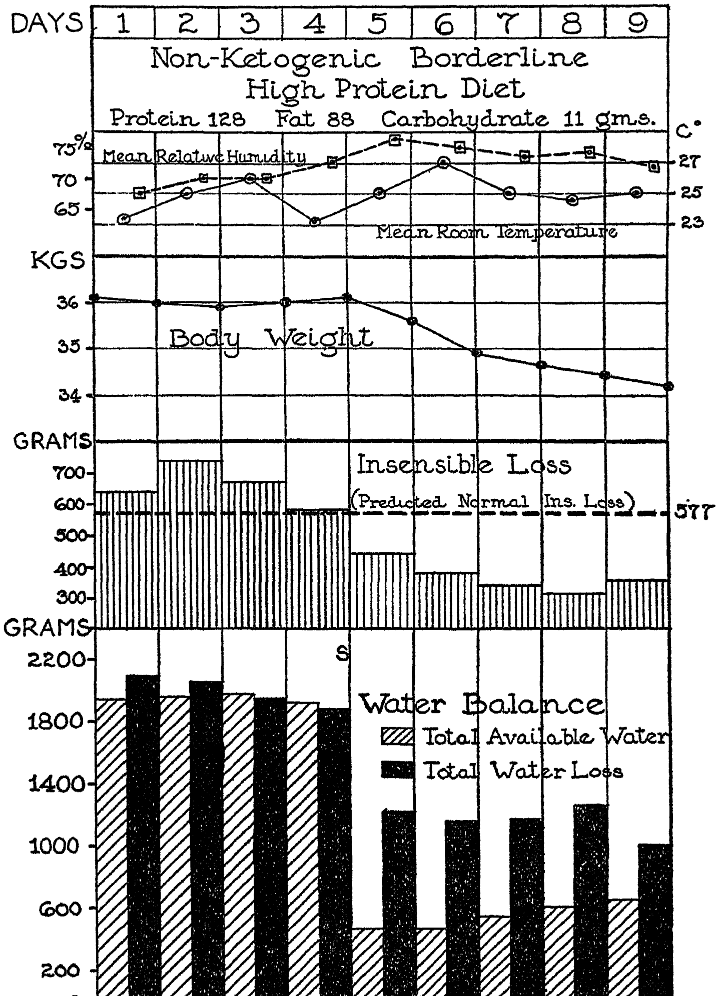

CHART 1.—Effect of marked dehydration from restriction of water intake on the insensible perspiration. "Predicted normal insensible loss" was estimated from the table of Benedict and Root according to the subject's calculated heat production. E. M., age 11 years. wt. 36 kg.

drating effect in the case of this particular patient so long as the total water intake was not restricted.

An examination of the data shown on Chart 2 reveals several interest-

ing points. The dehydrating effect of the ketogenic type of diet is clearly demonstrated by the results for the first nine days on which the diet was given. In spite of a fairly liberal water allowance, a sufficient degree of dehydration was produced to cause a fairly marked decrease in the insensible perspiration. In contrast to this, the substitution of an isocaloric, high-carbohydrate diet with the same total water content, was followed by rehydration of the tissues and a gradual increase in the insensible pers-

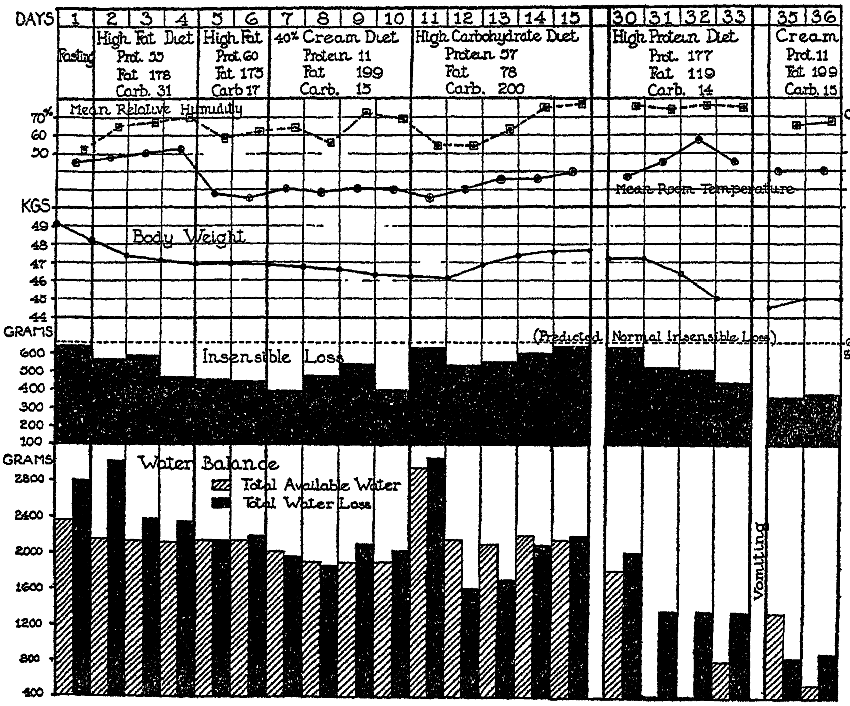

CHART 2.—Comparison of the effect of different types of diet and of different levels of water intake on the water balance and the insensible loss of body weight. W. J., age 14 years. wt. 49 kg.

piration. The marked dehydration which followed the use of a high-protein, non-ketogenic diet and stringent water restriction was likewise accompanied by a progressive decrease in the insensible perspiration. It is worthy of note that the absolute decrease was not so great as that observed during the subsequent ketogenic diet period, in which the degree of dehydration was perhaps no more intense. One possible explanation for this difference may be found in the greater specific dynamic action of the high protein diet. An increase in the total energy metabolism due to this factor would be expected to manifest itself in this way.

The relationship of the insensible perspiration to the state of hydration of the body is again shown in Chart 3. The data presented indicate that there is a rough parallelism between the degree of dehydration and the magnitude of decrease in insensible loss.

The effect of rehydration is also shown in the first and last periods of the experiment. The point of unusual interest in the first period is that relat-

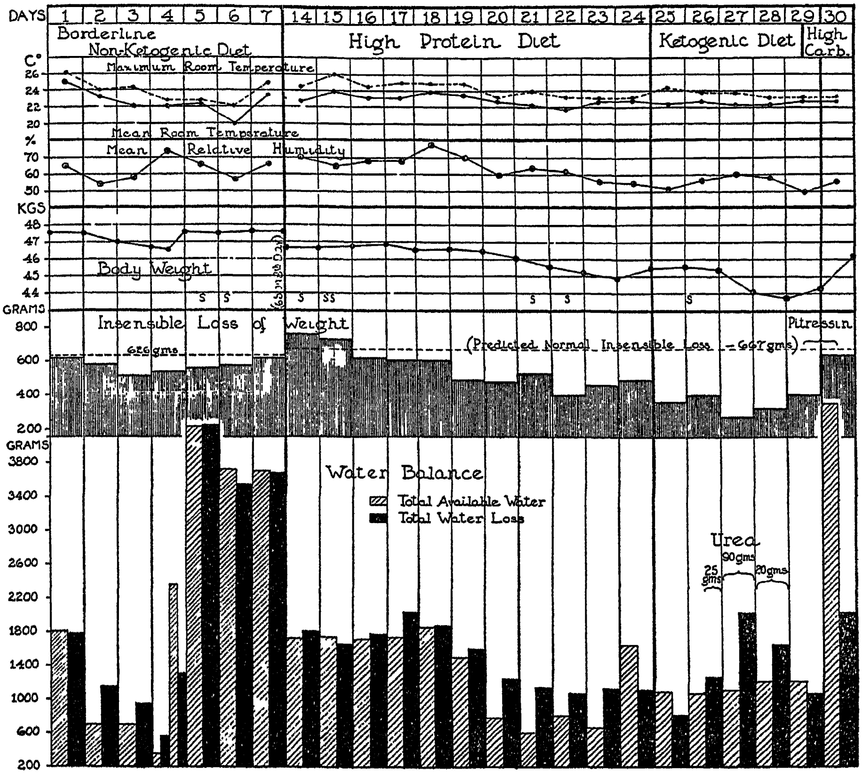

CHART 3.—Influence of different types of diet, of different levels of water intake and of diuresis on the water exchange and the insensible loss. J. R., age 16 years. wt. 48 kg.

ing to the time required for the insensible perspiration to return to its original value after completion of rehydration, as indicated by recovery of body weight. A satisfactory explanation for the observed delay when extra water alone was given is not apparent. It may indicate that the skin and subcutaneous tissues are rehydrated more slowly than the remainder of the body under these conditions in which the total sodium content of the ingesta was low. When rehydration was effected on the 30th day of the

TABLE I

Diet (1712 cal.)	Mean Room Temp. C°	Mean Relative Humidity Per cent	Body Weight KGS	Net Change in Body Water (gms.)	Degree of Dehydration	Total Calories for Period (12) hrs.			Insensible Perspiration for Period (12 hrs.)		
						Det'd (Tissot)	Predicted from Ins. loss	Percentage Difference	Meas-ured	Predicted from Tissot	Percentage Difference
Borderline Non-Ketogenic	24	70	47.41	0	None	659	657	− 0.3	326	327	− 0.3
	22	73	46.55	− 914	Moderate	639	580	− 9.2	266	312	−14.7
	23	66	47.60	+ 259	None	630	650	+ 3.2	319	310	+ 2.9
Ketogenic	22.5	54	46.62	0	Slight	672	654	− 3.2	322	340	− 5.3
	22.5	58	45.36	−1308	Fairly Marked	666	560	−16.0	251	331	−24.1
High Protein	23.5	68	46.46	0	Moderate	693	614	−11.4	293	350	−16.3
	22	72	46.75	+ 300	Slight	694	630	− 9.0	303	350	−13.4
	22.5	61	45.42	−1233	Fairly Marked	664	500	−24.7	205	330	−38
	22.5	51	44.53	−2087	Fairly Marked	655	518	−21.0	220	324	−32
	23	50	43.90	−2752	Fairly Marked	639	508	−21.8	212	312	−32

TABLE I.—Comparison of values for heat production as determined gasometrically and by the insensible loss method under normal conditions and in dehydration. Insensible perspiration as actually measured and as predicted from gasometric metabolism measurements compared. J. R., age 16 years. wt. 48 kg.

experiment by the simultaneous administration of water and the anti-diuretic hormone of the pituitary gland (pitressin) at regular intervals, recovery of the insensible perspiration from an extremely low level to an approximately normal one was very prompt. No conclusions can be drawn from a single observation of this kind, but it suggests a new angle of approach to the problem regarding extra renal regulation of water metabolism.

An extreme degree of dehydration was produced between the 25th and 29th days by the combined effects of a ketogenic diet, moderately stringent water restriction and administration in large doses of the diuretic substance, urea. On the third day, when 90 grams of urea were given, the insensible loss fell to 265 grams in 24 hours or 11 grams per hour, a level 60 per cent below the predicted normal value. In spite of the fact that on this day only 9.5 per cent of the body heat, instead of the usual 24 per cent, was dissipated through the invisible vaporization of water, there was no rise in body temperature. It is probable, however, that the degree of dehydration which results in a rise of body temperature is not far beyond this point.

The next experiment to be described was designed to ascertain whether the decrease noted in the insensible perspiration was due to dehydration *per se* or to a lowered energy metabolsim. Gasometric measurements of the metabolic rate were made at 2-hour intervals for 12 hours, beginning at 7:00 A.M. The total heat production for the period as determined by this method was compared with that predicted from the simultaneously measured insensible loss. As shown in Table I, the close agreement of values obtained under conditions of normal hydration, was entirely lacking during periods of dehydration. It is evident that dehydration of the grades observed in this experiment has no significant influence on energy metabolism and that decrease in insensible loss is relatively independent of the metabolic rate under such circumstances. The factor of respiratory quotient need not enter into the discussion here because the effect of water restriction was to reduce the insensible loss no matter what type of diet was given.

The effect of superhydration on the total insensible loss is shown by the data presented in Chart 4. In the experiment represented, the patient, a 9 year old boy, was given a basic diet of low-mineral content throughout the entire period, consisting of 200 grams of 40 per cent cream, 240 grams of cane sugar and 3600 grams of distilled water. This was divided into 4 equal parts, one fourth of each ingredient being given during the first three hours of each 6-hour period. Over certain periods, indicated on the chart

by brackets, pitressin, the antidiuretic principle from the hypophysis cerebri, or sodium chloride was administered separately or simultaneously.

Unfortunately, the insensible loss was not determined with the patient on a normal water intake. However, comparison of the values obtained for 12-hour periods on the regimen of high water intake with that estimated

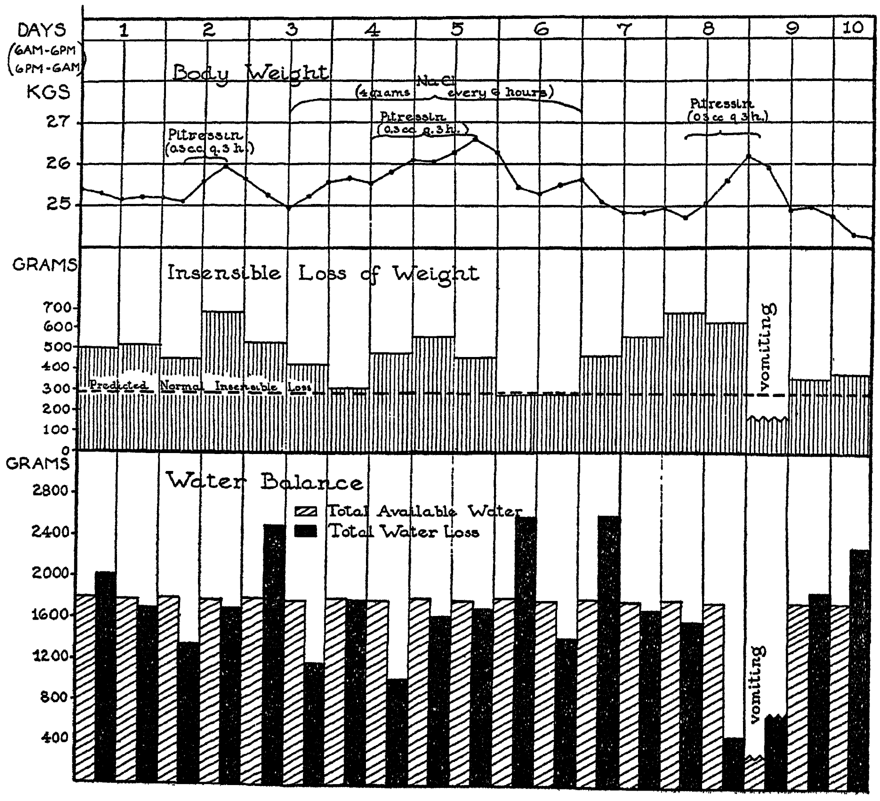

CHART 4.—Effect of "superhydration," as produced by forced water drinking and pituitary antidiureis, on insensible perspiration. Diet low in protein and minerals. Effect of added NaCl. N. F., age 9 years, wt. 26 kg.

from the table of Benedict and Root, for the same subject, shows that there was a marked increase throughout the period of study except for three of the days during which sodium chloride was being given without pitressin. With the administration of pitressin at three hour intervals, the insensible loss rose to a high peak, but again fell off on withdrawal of the extract. When 16 grams per day of sodium chloride were given, the insensible loss fell to normal on the second day, while the body weight showed an increase.

Upon resumption of the pitressin administration during the NaCl period, the body weight ascended to its highest point and the insensible loss was elevated though not to so high a level as previously. With discontinuence of the pitressin the insensible loss again returned to normal. Omission of the salt was again followed by a great increase in the insensible loss. Administration of pitressin accentuated this increase.

Discussion

The foregoing experimental data furnish proof of a definite relationship between the state of hydration of the body and insensible water loss. Dehydration, whether produced by a ketogenic diet alone, by restriction of water intake on a variety of diets, or by marked diuresis, resulted in a significant decrease in the insensible perspiration amounting in one instance to as much as 60 per cent. That the insensible loss, and not the total energy metabolism, varies under the conditions of our experiments is clearly shown. Although the CO_2 output was determined in but one case, it is practically certain that the changes in insensible loss under these conditions are due solely to changes in water output. Dehydration fever probably does not occur until the percentage of body heat dissipated by evaporation falls below 10 per cent under the conditions of our experiments. Increase in the insensible loss was consistently found to occur when large amounts of distilled water were given with a cream and sugar diet. This was still further accentuated when the antidiuretic extract (pitressin) of the hypophysis cerebri was administered in addition. Although the body weight increased when sodium chloride was given at the same time with the water, the insensible loss tended to return to the normal level. This result suggests that the amount of water lost insensibly depends to a certain extent upon the way in which it is held in the body. Further investigations should be made in this particular field.

In addition to their bearing upon the general subject of the water exchanges in the body, the results presented here have some practical importance. They demonstrate the necessity for having the tissues in a normal state of hydration ("saturated"), when the insensible-loss method is used for estimating the total energy metabolism. It is apparent also that, in addition to this requirement, extreme differences in type of diet and in mineral intake should be given consideration in the interpretation of results.

Certain of our epileptic subjects taking water *ad libitum* have been observed to develop fairly marked negative water balances and slightly nega-

tive sodium chloride balances on the ketogenic diets used in their treatment, while others, responding less well therapeutically, have not shown these effects. The insensible loss was diminished in the former cases. The reason for these differences in reaction to the diet is not clearly understood. Further investigation may show the necessity of regulating the mineral content of the diet, when metabolism determinations are to be made on persons following these unusual regimens. Under ordinary conditions, however, this precaution appears to be unnecessary, uniformly good results being obtained when the other requirements mentioned have been satisfied.

Slight alterations in the state of hydration probably do not add significantly to the errors inherent in the method of estimating metabolism from insensible loss. With a given set of conditions for any particular subject, however, the correspondence between the total insensible water loss and the state of hydration appears to be sufficiently uniform to suggest the use of net changes in body weight as an approximate measure of changes in state of hydration.

Summary and Conclusions

The total insensible loss of body weight or "perspiratio insensibilis" has been measured under standard conditons in five children ranging in age from 9 to 16 years. Variations, due to difference in diets, to restriction or forcing of water intake, to marked diuresis and to antidiuresis, have been determined.

In a normal ("saturated") state of hydration, there is a close parallelism between the insensible loss and the energy metabolsim under ordinary conditions. For practical clinical purposes, this method of estimating the energy metabolism, as proposed by Benedict and Root and amplified by Levine and Wilson and by Johnston and Newburg, offers many advantages over the ordinary gasometric methods.

Fairly marked dehydration from any cause interferes with the usefulness of the method, because the total insensible water loss is diminished to levels far below those indicated in the standard tables. Superhydration, due to excessive water drinking and to administeration of the antidiuretic principle from the hypophysis cerebri (pituitrin or pitressin), likewise interferes because in this state the values for the insensible loss may be far above those predicted from the tables. The type of diet and the mineral content of the ingesta apparently influence the insensible loss also and should be considered in the interpretation of results, when the regimen is

undergoing sudden changes or is extreme in any respect. Further investigation on this particular phase of the subject is urgently needed.

Degrees of dehydration, such as those reached in the present study, are not followed by significant alterations in the metabolic rate.**

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** The authors are indebted to Dr. L. H. Newburg for valuable suggestions regarding the technique of measuring the total water exchanges over long periods of time.

GROWTH AND REPRODUCTION ON MILK DIETS*

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ATTEMPTS to utilize cow's whole milk as the sole diet of animals have failed because of its inability to support growth and well-being. Young rats, for instance, when kept on such a diet develop a severe anemia in the course of a few weeks (1, 2). However, the addition of small amounts of the salts of iron and copper effectively prevents or cures such an anemia (3, 4). These findings have led us to study further the ability of cow's whole milk, when supplemented with copper and with both iron and copper, to support long continued growth and reproduction in rats. A study of both growth and reproduction is of course a much more exacting test of nutritive completeness and might reveal further deficiencies, if such existed.

EXPERIMENTS WITH MILK AND COPPER

In our first experiments, young rats were fed whole milk supplemented with copper alone. This was done to determine just how serious, from a nutritional standpoint, is the deficiency of iron. Three groups were taken at weaning and placed in cages with screen bottoms and fed milk supplemented with three different levels of copper, as copper sulfate. Group I, consisting of two males and four females, received 0.1 mg. Cu per 100 cc. of milk; Group II, consisting of 6 females, received 0.5 mg. Cu per 100 cc. of milk; and Group III, made up also of 6 females, received a supplement of 1.0 mg. Cu per 100 cc.

Hemoglobin determinations were made on the blood of these animals during the first few months either every week or every two weeks. Later the hemoglobin was determined at 4-week intervals.

In addition to the three groups of animals mentioned above, two other groups were available for a comparison of growth and reproductive performance on a similar diet, as part of an experiment designed to test the

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toxicity of copper added as copper sulfate. At the start of this experiment, 4 groups of four rats each were taken at weaning and fed different levels of copper. In the first group each rat received 0.25 mg. Cu daily, in the second 1.0 mg. Cu; in the third, 4.0 mg. Cu; and in the fourth, 16.0 mg. Cu. The animals of each group were kept in one large cage with a screen bottom and were fed the total copper allowance for the group in a small amount of milk in the morning. This was done in order that complete consumption would be secured. As this was consumed, more milk was added later. Hemoglobin determinations were not made on any of these animals.

The animals of the two groups receiving the highest copper dosages, namely 4 and 16 mgs. daily, did not long survive. Seven of the eight animals died in from two to four weeks. One of the rats which was receiving 4 mg. of Cu daily lived, however, for over 30 weeks, during which time it made slow but definite growth increases. It was finally discarded. These results indicate that young rats have, apparently, a large tolerance for copper, even when it is added to their diet in the form of copper sulfate. It is to be noted that the copper was offered to the rats in a rather concentrated mixture in the early part of the day and that, had it been mixed with a large quantity of milk so that it could have been consumed over a longer period, the tolerance might possibly have been higher. In other experiments in this laboratory, where a copper salt was mixed intimately with a dry ration, rats have consumed approximately 4 mg. daily over a period of 28 weeks and have grown and reproduced with every appearance of normality. It would, therefore, appear that copper is not so toxic as has been indicated often in the literature.

The two groups in this series which received the lower dosages of copper (0.25 mg. and 1.0 mg. Cu per animal daily), survived for long periods. Their performance will be discussed with the three groups described earlier. We will refer to them as Groups IV and V respectively. Group IV consisted of 2 males and two females; group V of 1 male and three females.

It is not our intention to discuss in any great detail the performance of these five groups receiving cow's whole milk supplemented only with copper, since it is known that the diet was otherwise deficient, as, for example, in iron. We shall summarize the results as briefly as possible and present typical instances in Charts 1 and 2.

It was surprising that the animals performed as well as they did. As might be expected, a marked anemia was exhibited which was of long duration in most cases. Only after many weeks, when the growth of the animal had largely ceased, did the hemoglobin approach anything like normal levels. In many instances it remained below normal throughout the

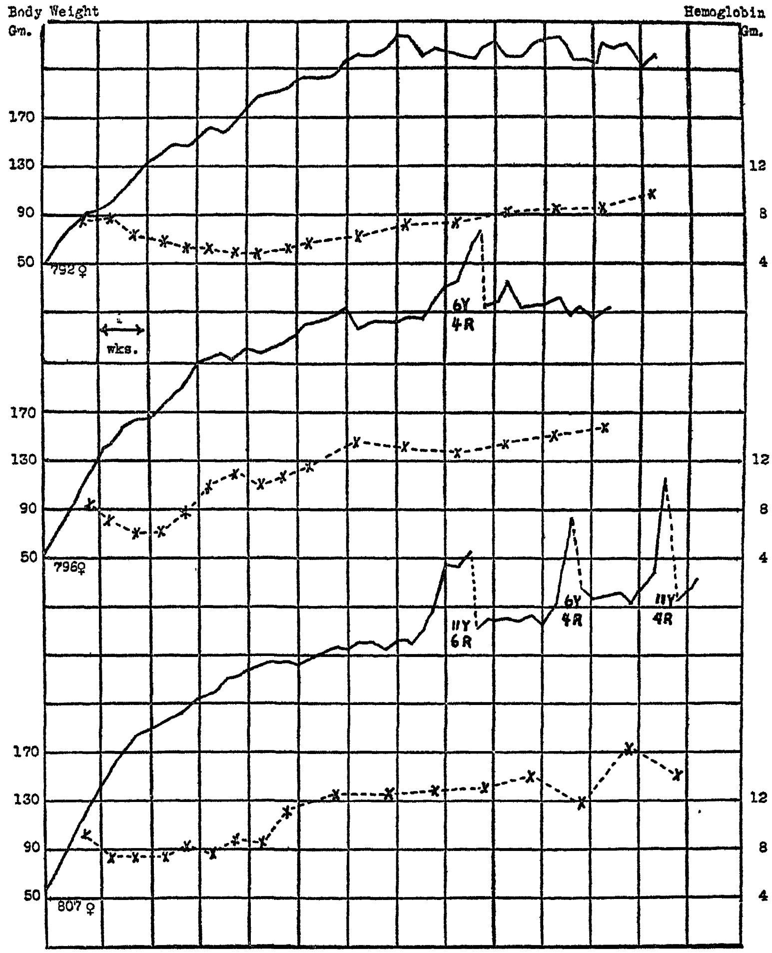

CHART 1.—PERFORMANCE OF FEMALE RATS ON A DIET OF WHOLE MILK AND COPPER. Slow growth and long continued anemia may be noted.

No. 792 is one of the group that received 0.1 mg. Cu as copper sulfate per 100 cc. of milk. In this group there were 4 females, one of which had one litter of 3 young after over 40 weeks on this diet. The other females produced no young.

No. 796 is one of the group that received as an addition to the whole milk diet 0.5 mg. Cu as copper sulfate per 100 cc. of milk. In this group of six females only one other besides No. 796 produced young, a litter of six being born to each. No. 796 was the only one to raise her young to weaning.

No. 807 had the best record in the group of six females that received copper at the level of 1.0 mg. Cu per 100 cc. of milk. Three others produced one litter each. No. 807 raised some of each of her litters.

In this and the charts that follow the solid line represents body weight while the broken line with X's are hemoglobin values in gm. per 100 cc. of blood. The number and letter Y indicate the number of young born and the number and letter R indicate the number of young raised to weaning.

life of the animal. The growth responses of the animals were sub optimum but good weights were obtained eventually, especially in those groups receiving the higher dosages of copper (1.0 mg. Cu per 100 cc. milk and 1.0 mg. Cu per animal daily). In the group receiving the lowest copper supplement (0.1 mg. Cu per 100 cc. of milk), the two males attained weights of 290 to 300 grams after 28 weeks on the diet. Females in this same group (in all but one case) attained weights of 240 gm. or over in 26 to 28 weeks. However, hemoglobin values remained quite low, never getting above 10 or 11 gm. per 100 cc. of blood and being generally less than 7 gm. during most of the lifetime of the rats. Four of the animals succumbed to infection of the respiratory tract after 40 or more weeks, and the other two were discarded after more than a year on the diet. Growth and well being were invariably better in the other four groups.

Opportunities for mating were provided for all the females in these groups either with males in the same group or with stock males which were placed in their cages. When stock males were used these were changed at intervals so that they did not have to exist for extended periods on the milk and copper diet. Females in advanced pregnancy were segregated in individual cages provided with shavings as litter. They were returned to their groups after their young were weaned or had died. Living young were born in all groups. A total of seventeen litters was secured from the females of the five groups during the period of 47 to 55 weeks that they were kept on this diet. The poorest reproduction was shown by Group I which received the lowest level of copper. Among the four females of this group only 1 litter of three young was born. In all groups except one there were always some females which produced no young while other females produced two or three litters.

In view of the fact that it has been suggested that milk is low in vitamin E, we were on the watch for any evidence of resorption of young but found no indication of it in any instance. To further test this point we made daily vaginal smears on two females of Group IV that, although well grown and apparently vigorous, had not produced young as had the other female in this group. For a period of about 160 days vaginal smears were made daily to detect ovulation and any mating. During that time ovulation was very irregular, one female having a dioestrum period at one time of 85 days while the other went for 66 days without ovulating. No evidence of mating was ever found even at such times as they did ovulate, although the male in the group was known to be fertile. It seems, therefore, that the lack of fertility in some of the females on this diet must be ascribed to some other

disturbance than that of resorption of fetuses. This point will be discussed in subsequent pages.

The mammary function of females on this diet of whole milk and copper was also poor. Many of the litters born were not suckled, and they died in the course of a few days. Some individuals from nine of the litters were, however, suckled and raised and were eventually weaned and placed on the same diet that their dams had received. Representatives of all the groups except Group I were present in the second generation. The poor mammary function of the dam was further shown in the slow growth of these young during the suckling period. When weaned, between three and four weeks of age, they weighed generally from 38 to 44 grams which is 10 to 12 gm. below the weight of young of the same age from our stock colony. It was noted also that for some time before they were weaned they were getting most of their nourishment from the dam's feed dish and not by suckling.

It must, however, be emphasized at this point that nothing in the way of paralysis, or screaming or running fits was ever noted in any of the young from these milk fed animals. These symptoms have been observed by others (5, 6) in young suckling rats and have been explained as being caused by the specific lack of vitamins E and B. We observed no such symptoms.

The second generation consisted of 20 females and 20 males divided between 9 litters. Some of the earlier of these litters were maintained for periods of 51 to 52 weeks on the identical diets that their dams had received. Other of the later litters were discarded after about 40 weeks. Opportunities for mating were provided as all litters contained individuals of both sexes.

The growth and reproduction of the second generation was practically as we have described above for the first generation—hence no additional discussion is necessary. A total of 7 litters of young, most of which were alive at birth, was born. Of these, 4 litters were nursed more or less successfully, so that altogether 12 individuals were weaned and placed on identical diets, representing the third generation.

In Chart 2 are presented the records of two of these third generation animals (No. 1499 ♀ and No. 1454 ♂). Long records are not available on any of the individuals, of this generation. As may be seen, however, initial growth is fair in spite of the marked anemia. Vaginal smears were made on the females and the ovulations of No. 1499 are indicated on the chart. These are at a rate much below normal. It may be noted that the male, No. 1454, was shown to produce viable sperm since he sired two small litters of young, none of which, however, lived.

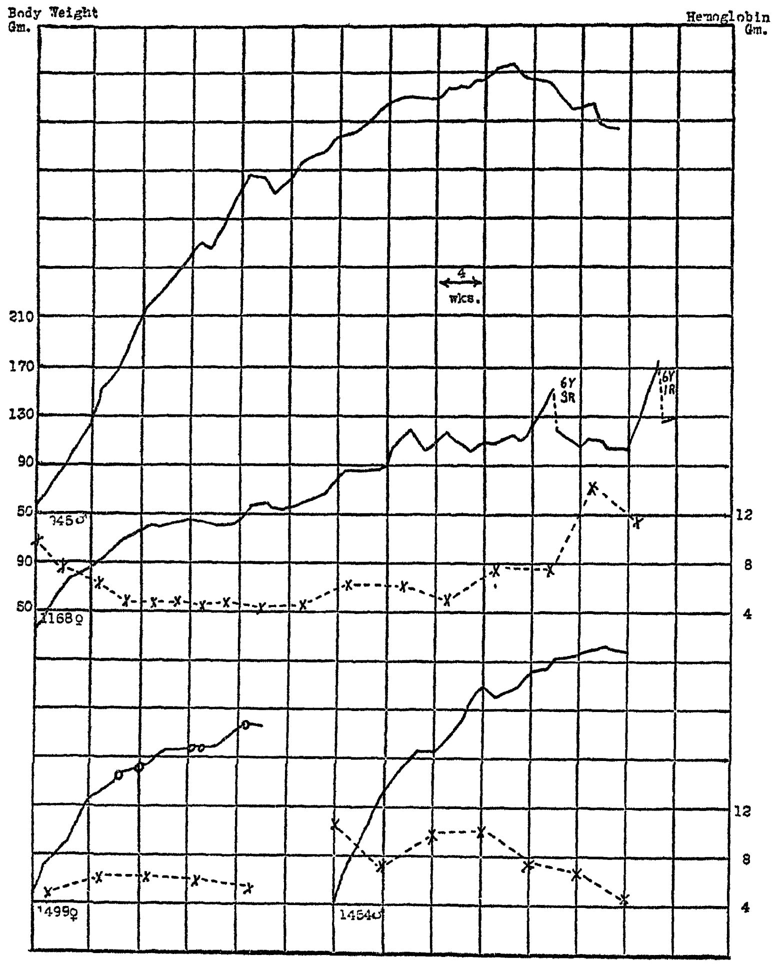

CHART 2.—GROWTH AND REPRODUCTIVE PERFORMANCE ON A DIET OF WHOLE MILK AND COPPER.

No. 945 received 1.0 mg. Cu daily in addition to his diet of cow's whole milk. Growth as may be noted was good. He was one of a group consisting of three females besides himself. One of the females produced three litters of 9, 9, and 5 living young, of which he was the sire. The last litter was born one week before he was discontinued. He attained to a greater weight than other males on similar diets.

No. 1168 was one of a group of five second generation animals (2 ♂ and 3 ♀). They received 0.25 mg. Cu daily per animal in addition to milk diet. One of the ♂s died after 33 weeks and one of the ♀s after 38 weeks from pulmonary infection. The other female, besides No. 1168, produced one litter of 6 young of which she weaned 3. As instanced by 1168, growth was poor and anemia was marked.

Nos. 1499 and 1454 are two representatives of third generation groups on milk and copper. No. 1454 was known to be the sire of two small litters of living young.

In this and the following charts the O's on the growth curve indicate ovulations.

Experiments on Milk Supplemented with Copper and Iron

During the past year we have carried on experiments (in addition to those described above) in which the milk diet was supplemented not only with copper but also with iron. In view of our experience with the performance of animals on the copper supplemented milk, it was assumed that the further addition of iron might improve the diet so that better growth and better reproduction would result. It was also thought that, should the iron and copper supplemented milk diet still prove deficient, the possibilities of discovering the nature of these deficiencies would be greater when both the inorganic elements, *viz.*, iron and copper, were present in sufficient amounts.

The diet used was cow's whole milk supplemented with 0.5 mg. Cu as copper sulfate and 1.0 mg. Fe as ferric chloride per 100 cc. of milk. In one group a slight variation was made in that the iron was fed at the rate of 0.5 mg. Fe per animal daily in a small amount of milk and as this was consumed more milk was added containing the above copper concentration. The animals were placed on the milk diet at weaning and kept in cages supplied with screen bottoms.

Both males and females were started on these diets either in groups by themselves or in groups where litter-mate brothers and sisters were present. Because of a marked testicular degeneration noted in males confined to this diet, a discussion of their performance will be set forth in another publication. The present discussion will be confined to the performance of the females.

A total of 19 females (first generation) on the milk, copper, and iron diet was studied. We present typical instances of their performance in Chart 3 in which are shown growth, hemoglobin values and reproductive performance. In brief, it may be said that the growth was of the same order as that secured in the previous experiments in which copper only was added to milk. Good weights were eventually secured, but neither the increases in weight nor the final weights attained were optimum. The hemoglobin values remained fairly uniform throughout and no marked anemia was present, but the values remained close to 12 gm. per 100 cc., which is probably close to the lower limits of normal values. Probably due to the absence of the anemia, less evidence of infections of the respiratory tract and a slightly better condition in general was observed in these females as compared with those in the earlier experiments on milk and copper.

Among the first animals to be started on this diet were 6 females which, after they were well grown, were given the opportunity to mate with males

on a similar diet. Following a lengthy period in which there was no evidence of mating or pregnancy, they were mated with stock males and daily vaginal smears were made on them in order to gain more insight into their reproductive behavior. The taking of vaginal smears was started about 20 weeks after they had been on the diet and was continued until they were discarded. Three of the females were carried along on the diet for a total period of 56 weeks; two, because of very poor ovulation, with absence of mating, and rather poor general condition, were discarded after 45 weeks; and the sixth one died after 27 weeks on the diet. In females that mated and became pregnant, vaginal smears were discontinued after the implantation sign (R.B.C.) was observed. Just before parturition, the females were segregated individually in cages provided with shavings as litter and allowed to bear their young and suckle them if they could do so. When the young were either all dead or else weaned, the females were returned to the group and daily vaginal smears made again.

Of the above six females, three mated and bore young. One had three litters of 8, 9, and 6 young but raised only 6 of the first litter. The second had one litter of 6 which she did not nurse, while the third had a total of five litters of which she successfully raised three. This last individual is No. 1262 of Chart 3. Poor mammary function was evidenced here again in the lack of ability to nurse the young and in the slow growth of the young that were raised. The young rats were considerably below the weight of young from our stock colony at the regular weaning age of 3 to 4 weeks.

The litters which were raised were continued on the same diet. The majority of these animals were, however, males and very little data are available on the performance of second generation females. One such female produced one litter of 7 young which she did not raise. She had been on the milk, iron and copper diet for about 22 weeks when her litter was born. Growth in the second generation was not so good as in the first.

Other females of first generation groups, started later than the one discussed above, had similar histories. Not all of the 19 females (first generation) kept on this diet had the opportunity to mate, because of the fact that we wished to study more thoroughly the ovulatory rhythm without the disturbance of mating. In the last group started on this diet, a group of 7 females, observations were made to determine the time at which the vaginal orifice opened. As soon as the orifice opened, daily vaginal smears were made. In one of these females the vaginal orifice opened after 7 weeks on this diet (age 78 days), in another it opened after 9 weeks (age 91 days), in two others it opened between 10 and 11 weeks after being started on the

diet, in the fifth animal it did not open until after 15 weeks on the diet, while in the remaining two animals the vaginal orifices have not opened to date (22 weeks on the diet—age around 175 days). Long and Evans (7) have shown that the vaginal orifice is established in normal females at an average age of 76.5 days.

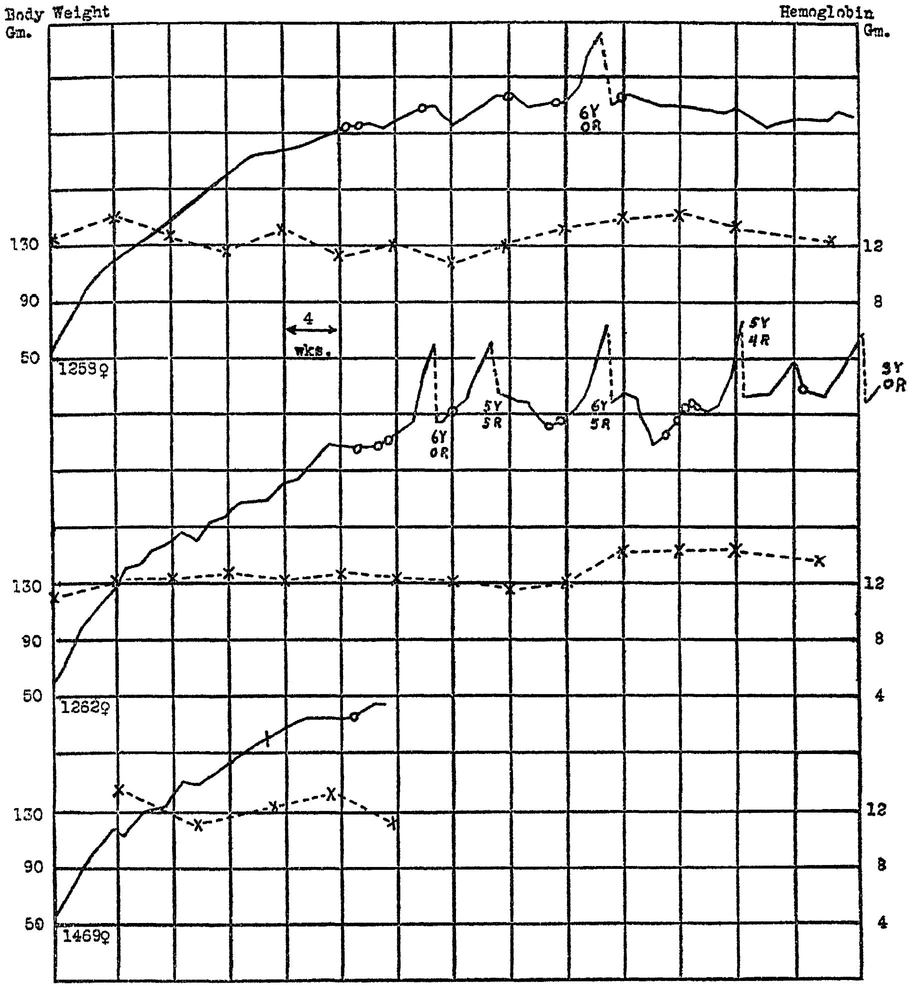

CHART 3.—PERFORMANCE OF FEMALE RATS ON A DIET OF WHOLE MILK, IRON, AND COPPER.

Nos. 1258 and 1262 are two of a group of six females, three of which did not reproduce. The performance of No. 1262 is the best we have encountered on milk diets. The | on the curve of No. 1469 indicates the time at which the vaginal orifice opened. Ovulations were slow and irregular on this diet.

In those animals in which the vagina opened naturally, ovulation has been very poor as is shown in Chart 3 (Rat No. 1469). This poor ovulation has been noted in all females on this diet on which vaginal smears were made. Even in the case of No. 1262, which made such an exceptional record, the early ovulations were at a very slow rate.

Because of this exceptionally poor ovulatory rhythm we made some preliminary experiments in which we attempted to improve the ovarian func-

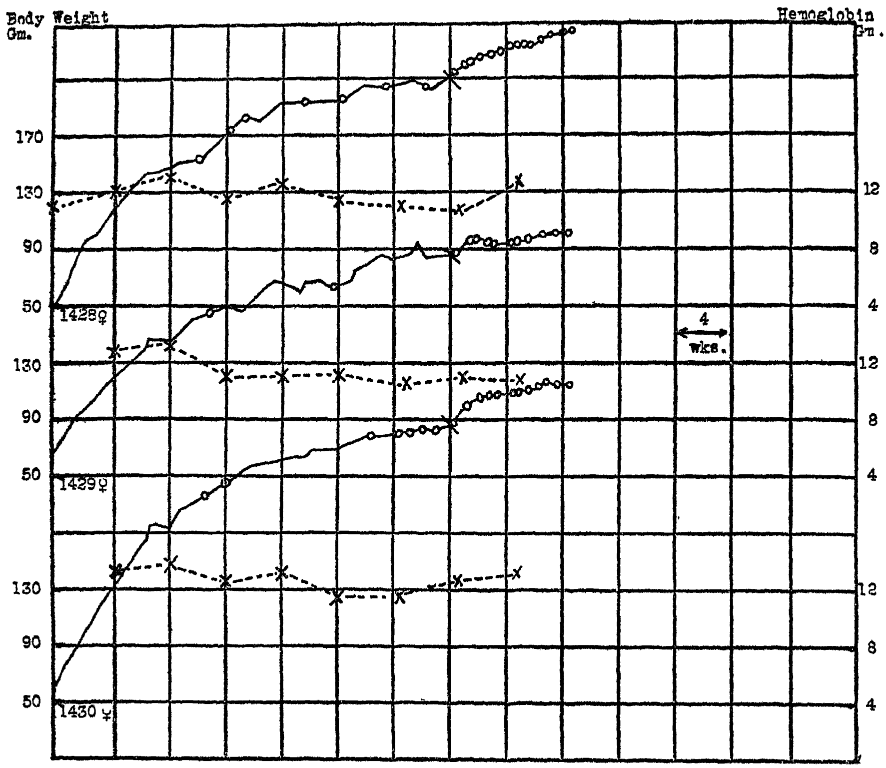

CHART 4.—THE EFFECT OF MANGANESE AND IODINE ON INCREASING THE OVULATORY RHYTHM. At the point indicated by \ additions were made to the basal diet of milk, iron, and copper as follows: No. 1428 received 0.5 mg. Mn per 100 cc. of milk; No. 1429 received 0.2 mg. I per 100 cc. of milk; while No. 1430 received both these additions. Later the iodine dosage was reduced to 0.002 mg. I.

tion by the addition of small amounts of inorganic substances. The known effect of low vitamin B intake on suppressing ovulations (8) and the slow growth of the young suggested that the milk diet might be low in vitamin B. On the other hand, it did not seem to us, in view of the continued production of young in several generations with the absence of any specific dis-

turbance in them during the suckling stage, that vitamin B could be low. Consequently we attempted to improve the diet by the addition of inorganic substances only.

The two elements which first occurred to us as possibly being present in insufficient amounts were manganese and iodine. Manganese has been found by Titus *et al* (9) to supplement iron effectively in curing the nutritional anemia produced by a milk diet and, while we could not confirm these findings in our laboratory (10), we have kept in mind that it might be found to be necessary or beneficial when animals were kept on milk for longer periods. Iodine was suggested to us by the fact that Maurer and Ducrue (11) have shown that, next to the thyroid, the ovary and uterus among the organs of the body possess the most iodine.

In view of these considerations we decided to add these elements to the diet of milk, iron, and copper. Of the 19 females discussed above, we chose three which had been on this diet for about 28 weeks and which had ovulated only at long intervals. To the diet of one was added manganese as a solution of manganese chloride at the level of 0.5 mg. Mn. per 100 cc. of milk, to another iodine as a solution of KI at the level of 0.2 mg. I per 100 cc. was added, while the third female received both manganese and iodine in the above concentrations. Later the iodine dosage was reduced to 0.002 mg. I per 100 cc. Immediately following either addition, the females began to ovulate at very regular intervals, namely every 4 to 5 days, and have continued to do so. We present in Chart 4 the details of this experiment.

Discussion

As we have already stated, our object in carrying out these experiments was to obtain more information on the nutritive properities of cow's whole milk. Our observations have covered the field of toxicity of copper, growth, ovulatory rhythm, ability to bear and nurse young on a sole diet of milk supplemented with small amounts of copper and with small amounts of copper and iron. Our results have indicated, that, for the rat at least, milk so supplemented is still not a perfect food. Growth, as we have shown, is not at a normal rate. This we assume, in the absence of more specific information, may be due to a low intake of calories because of the large amount of fluid that has to be ingested and that a limiting factor may be the capacity of the animal to handle sufficient quantities. In preliminary experiments in this laboratory we have doubled the caloric content of the milk by adding, among other things, whole milk powder, and have observed much better rates of growth in the early weeks. Whether this is entirely due to an increase of calories remains to be seen.

The poverty of milk in certain specific vitamins, notably vitamin B, has been the subject of much discussion. Not wishing to go into a complete discussion of the literature, we would refer to a recent article by Daniels, Jordon and Hutton (12) in which they deal with this subject and its relation to the growth of suckling young in milk fed rats. We prefer to explain the slow growth of young born to our milk fed females as being due to poor mammary function in the mother (quantitative) and not to any lack of known vitamins in the mother's diet. We are influenced in this opinion by the total absence of any specific symptom in the young, such as muscle incoördination, screaming or running fits, or collapse, and the fact that young, showing evidence of being nursed at all, grew and were weaned even though the growth rate was slow. It is noteworthy that certain females (see 1262) were able to produce several litters of young and nurse them more or less successfully on this diet without themselves or the young showing distinct evidence of lack of vitamin B.

The decreased lactating ability of the females we are unable to explain unless it is that the low caloric intake does not allow full mammary development. It is altogether possible that the decreased mammary function is another expression of deranged ovarian activity, as are the irregular and slow ovulations observed on these milk diets.

The taking of daily vaginal smears on all of the later animals has given us a better insight into the reasons for the poor reproduction of these milk-fed females. It is apparent that sexual maturity comes much later on this diet than in normal animals. Ovulations, even when once initiated, come generally at very long and irregular intervals and mating is not always accepted during these ovulation periods. The exact nutritive deficiency that is responsible for this condition we cannot yet decide, but the results so far secured with the addition of small amounts of manganese and iodine suggest that these inorganic elements may play an important part in the normal acitivity of the ovary and that they are not present in sufficient concentration in the milk which we used. It is of interest in this connection that Daniels and Hutton (13) secured better reproduction in milk fed animals by adding certain inorganic materials which included manganese and iodine.

Our study of the reproductive performance of milk-fed females has shown that there is no evidence of lack of vitamin E in animals subsisting on the whole milk used by us. This is of importance in view of the fact that milk has been thought to be low in this factor. In no instance have we found any evidence of resorption of young in pregnant females. Our experience has been that in those females in which mating and pregnancy had

been established by vaginal smears, young were always delivered. This is of further interest when it is considered that a marked testicular degeneration has been noted in males confined to a diet of milk, copper, and iron. These findings will be reported in another publication.

Summary

A study has been made of the growth and reproductive performance of rats on diets of cow's whole milk supplemented with copper and with copper and iron.

On a diet of whole milk and copper a chronic anemia, due to the low iron intake, was observed. Growth and reproduction were below normal, although fair weights were eventually reached and living young were produced. Many of the young born were not nursed, and those that were suckled grew more slowly than young from our stock colony. Third generation animals were secured.

On a diet of whole milk, iron, and copper, no anemia was observed but growth and reproduction were still sub-normal. The rearing of young was poor, due to poor mammary secretion. It is suggested that subnormal growth and poor rearing of young may be due to the low caloric diet. No specific evidence of low vitamin B was observed.

By means of daily vaginal smears it was established that subnormal reproduction was due to late sexual maturity and very poor ovulation. Some females ovulated at very long intervals and never mated. Preliminary experiments indicate that the addition of small amounts of either manganese or iodine, or both, greatly improves the ovulation rhythm in females on the milk, copper, and iron diet.

No evidence of lack of vitamin E was noted in females on these milk diets.

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MALE STERILITY ON MILK DIETS*

By

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IN THE immediately preceding paper we have discussed the effect of supplementing a diet consisting of cow's whole milk with copper and iron salts on growth and reproduction in the female rat. In this paper we wish to report the occurrence of male sterility on the same diets. We first noted testicular degeneration in two male rats on this diet in some of our early experiments when studying the supplementary effect of copper and iron on the cure and prevention of anemia caused by milk diets. In those experiments it was our custom to take young rats at weaning and put them on a sole diet of milk until a marked anemia had developed. When the hemoglobin level was sufficiently reduced, the rats were taken for experiments in which corrective measures were tried. Out of each of two groups of such animals that had received an addition of iron and copper to the basal diet, a male and a female were retained to determine if continued growth would take place and if they would reproduce.

The history of these four animals need not be detailed here, but it was eventually found that both males were sterile while one of the females mated with a stock male produced living young on this diet. One of the males died after having been on the milk diet for 39 weeks while the other male was continued for more than a year. In both cases evidence of sterility was noted as indicated by infertile mating and by the presence of non viable sperm in the vaginal plug. At death the right testis from each was saved for histological examination.

The testes were small and extremely edematous, and smears from the cut epididymis showed complete absence of sperm. Later histological sections of the testes showed complete degeneration as evidenced by the absence of germinal epithelium. Because of the fact that these animals had lived

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through an early period of severe anemia and because they had been on such low levels of copper intake that their hemoglobin levels remained subnormal, it was thought that the long continued anemic condition might have been responsible for the testicular degeneration. It was also thought, at that time, that the sterility might have been occasioned by the low vitamin E of the diet since many workers have brought forward evidence that milk is low in vitamin E. In later pages we will discuss our findings in this regard since we do not believe this to be the cause of the sterility observed in our experimental male rats.

In later experiments in which both male and female rats were made to subsist for long periods on whole milk supplemented with iron and copper, we again observed sterility in the males, characterized as before by the complete disappearance of the germinal epithelium. In nearly all of these experiments, iron was added in the form of a solution of ferric chloride at the rate of 1.0 mg. Fe per 100 cc. of whole milk, and copper in addition as a solution of copper sulfate at a level of 0.5 mg. Cu per 100 cc. of milk. These additions were made to the diet from the time that the animals were weaned and hence their hemoglobin level was maintained at approximately a normal figure. It would seem, therefore, that anemia, acute or chronic, was not an essential factor in the cause of this sterility. All of the first generation males on this diet grew well, reaching weights around 300 gm. after 16 weeks.

In the first of the later experiments a group of 4 males was kept on the above ration for 15 weeks after which the males were caged individually with females on the same diet. After some weeks, when no pregnancies resulted, daily vaginal smears were started on the females to determine if mating was taking place. A few days later a vaginal plug was found in one of these females but very few sperm could be found and those seen showed signs of abnormality as none were motile. The female did not become pregnant. Following this observation, it was decided to subject the males to test matings with young normal females on our stock diet. The normal females were subjected to daily vaginal smears and when any was found to be in the beginning stage of ovulation she was placed in the cage of one of the males. The following day search was made for a vaginal plug or residual sperm and the female was returned to her own cage. Test matings were secured on three of the males, but the fourth one never copulated although several opportunities were afforded. From these matings, pregnancy never resulted and spermatozoa were either abnormal in appearance, or absent from the vaginal contents.

The first infertile mating observed occurred when the males had been on the diet about 21 weeks at which time the male in question was 175 days old. In order to allow the degeneration to proceed to the maximum, the males were continued on the diet for several weeks longer. Two were killed after 35 weeks and the other two after 38 weeks. The right testis was fixed and saved for histological study while the other was used for determining the fresh weight and for making smears from the left epididymis. These animals are numbers 1263, 1264, 1265, and 1266 of Table I.

In a second group started on this diet three males were present. These were allowed to continue for 32 weeks after which they were killed; no mating history was secured on them. They are numbers 1325, 1326, and 1329 of Table I.

When it was apparent from the mating history of the first group that degeneration was to be expected, a slight modification was made in the diet of a later group. This group of six animals, littermates, made up of three males and three females, received iron at the rate of 0.5 mg. Fe each daily in a small amount of milk in the morning and after this was consumed they received more milk containing the same copper concentration as described above. This was done because it was the iron that was suspected of causing, either directly or indirectly, the injury to the testis. Therefore, by feeding it all in a small amount of milk, the major part of the diet was not subjected to its prolonged influence. The group was kept in one large cage supplied with a screen bottom.

Approximately 10 weeks after the animals were started, vaginal smears were made daily on the females. Two days later the remains of a vaginal plug were found in one of the females. This showed very few sperm. The sperm were abnormal, having swollen heads and being tangled in masses. No free sperm were found in the deeper portions of the vaginal canal, which would indicate that none of the sperm were motile. Twelve days later the same female mated again, and again abnormal sperm were found in the vaginal contents. The female did not become pregnant and ovulated 10 days later. This indicated that even as early as 10 weeks on this diet marked degeneration was present in the testes of these males. Immediately following this one of the males (No. 1425) was killed and his right testis fixed for histological study. The other two were separated from the females for further testing with young, normal females.

Two such test matings were secured during the next few weeks with two young females from our stock colony. In both cases the sperm found in the vaginal canal gave evidence of abnormality, and in no case did either fe-

TABLE I
TESTICULAR DEGENERATION ON DIETS OF MILK, IRON, AND COPPER

Rat no.	Initial weight	Final weight	Weeks on diet	Weight of testis (trimmed)	Condition of testis	Epididymal contents	Histological findings
	(gm.)	(gm.)		(gm.)			
1263	55	340	35		Very edematous	No sperm seen.	Germinal epithelium completely absent, tubules reduced in size.
1264	58	320	35	0.88	Very edematous.	Very few, very long, non-motile, D sperm, epoorly defined hads.	Same as 1263—complete degeneration.
1265	58	326	38	0.80		Very few sperm-like structures—unusually long with no heads — two fused together.	Tubules much reduced in size and also shrunken. No germinal epithelium.
1266	58	274	38	0.79		No sperm seen.	Same as 1265—no germinal epithelium.
1325	61	337	32		Edematous.	Few sperm. Non-motile—some headless, some had swollen heads.	Most of tubules denuded of germ. epithelium. Some few tubules possess spermatagonia. One or two tubules possess giant cells.

1326	58	353	32	0.81	Edematous.	Same as 1325.	Complete absence of germinal epithelium. Tubules small and misshapen.
1329	59	370	32			More sperm than 1325—non-motile, heads broken off, swollen heads.	Complete absence of germinal epithelium, same as 1326.
*1425	58	230	12	0.60	No marked edema	Some sperm—*one* seen moving in whole smear—others abnormal.	Germinal epithelium gone in all but few tubules. In these are giant cells.
*1427	58	185†	16			Large number of sperm—some motile, majority of sperm are clumped—"tufts of hay."	Many outside tubules show complete degeneration. Other in less advanced stage have giant cells. Some tubules in center are normal.
*1426	60	276	24	1.46		Many motile sperm apparently normal.	All tubules but 3 or 4 are apparently normal. These 3 or 4 show complete or almost complete absence of germinal epithelium.

* These three animals received iron dosage at rate of 0.5 mg. Fe daily in small amount of milk in morning and copper supplemented milk later. The other animals in this table received iron at rate of 1.0 mg. Fe per 100 cc. of milk.

† This rat weighed 233 gm. the week before he was killed. The drop in weight was occasioned by an infection in left nostril with swelling causing his nose to curve to right so that his incisors did not occlude. He ate very poorly during the last week.

TABLE I (continued)

TESTICULAR DEGENERATION ON DIETS OF MILK, IRON, AND COPPER

Rat no.	Initial weight	Final weight	Weeks on diet	Weight of testss (trimmed) (gm.)	Condition of testis	Fpididymal contents	Histological findings
1448	59	203	15	1.16	No marked edema.	Very many motile sperm.	Normal testis.
1447	51	190	18	0.53	Edema.	Few sperm but none normal and no motility.	Germinal epithelium disappearing from some tubules. In central tubules epithelium not differentiated. Epithelium deep but no sperm apparently produced.
1449	52	198	23	0.58	Very edematous.	No sperm.	Only 4 or 5 tubules show any germinal epithelium. Rest show complete absence of epithelium. All tubules small.
1446	50	205	25	0.8	Very edematous.	No sperm.	Absence of germinal epithelium, much edema, tubules small.
1450	52	192	25	0.55	Very edematous.	Few degenerate sperm. No motility.	75 per cent of tubules show almost complete absence of germinal epithelium. Remainder have more or less germinal epithelium—some having deep epithelium but little differentiation.

male become pregnant to these matings. It is to be noted that both males were in the same cage and it was assumed that both had participated in the copulations. Some time later one of the males (No. 1427) was killed because of an infection in his left nostril which interfered very much with his eating, and his right testis saved for histological sectioning. The remaining male (No. 1426) was continued on the same diet.

He was further tested about 4 weeks later with normal females and found to be apparently normal. The sperm seemed of normal morphology and females became pregnant. This was rather surprising but further tests proved that he was able to produce normal sperm because four litters of living young were sired by him. He was killed after 24 weeks on the diet and a description of the histology of his testis is reported in Table I.

The above described ten animals are all of the first generation male rats that have been so far examined on the iron and copper supplemented milk diets. We have in addition, however, had the opportunity to study a litter of second generation animals, produced on this same diet (1.0 mg. Fe and 0.5 mg. Cu per 100 cc. milk), by a female that was mated with normal males. It so happened that of the six raised by her, five were males. It was thought that the condition would be much more severe in second generation males, but our observations being limited to these five, are not sufficiently extensive to determine this point. In Table I we have presented the findings which were secured by killing the second generation individuals at varying dates (Nos. 1446–1450). It is apparent that they too succumbed to the testicular disease.

In Table I we present summarized post mortem findings on the animals which we have described above. When the animals were killed the right testis with its epididymis was dropped immediately into a fixing solution as a preparation for sectioning, the capsule of the testis being incised to hasten fixation. We used Bouin's fluid mainly, although we also used a formalin solution in some of the first work. The left testis was removed as soon as possible and the epididymis snipped across with sharp scissors and the cut surface smeared on a drop of Ringer's solution on a glass slide. This was examined immediately under the microscope. In turn, the left testis was trimmed clear from the epididymis and fatty tissue and weighed. In practically all cases the weights were much below normal. If one takes, for example, the first four animals of Table I it will be seen that the average weight of the trimmed left testis was approximately 0.8 gm. Donaldson (1) gives 2.694 gm. as a normal figure for the two testes of male rats of around 300 gm. in weight. This is a weight of 1.34 gm. each. The weights as recorded by us do not, however, give a

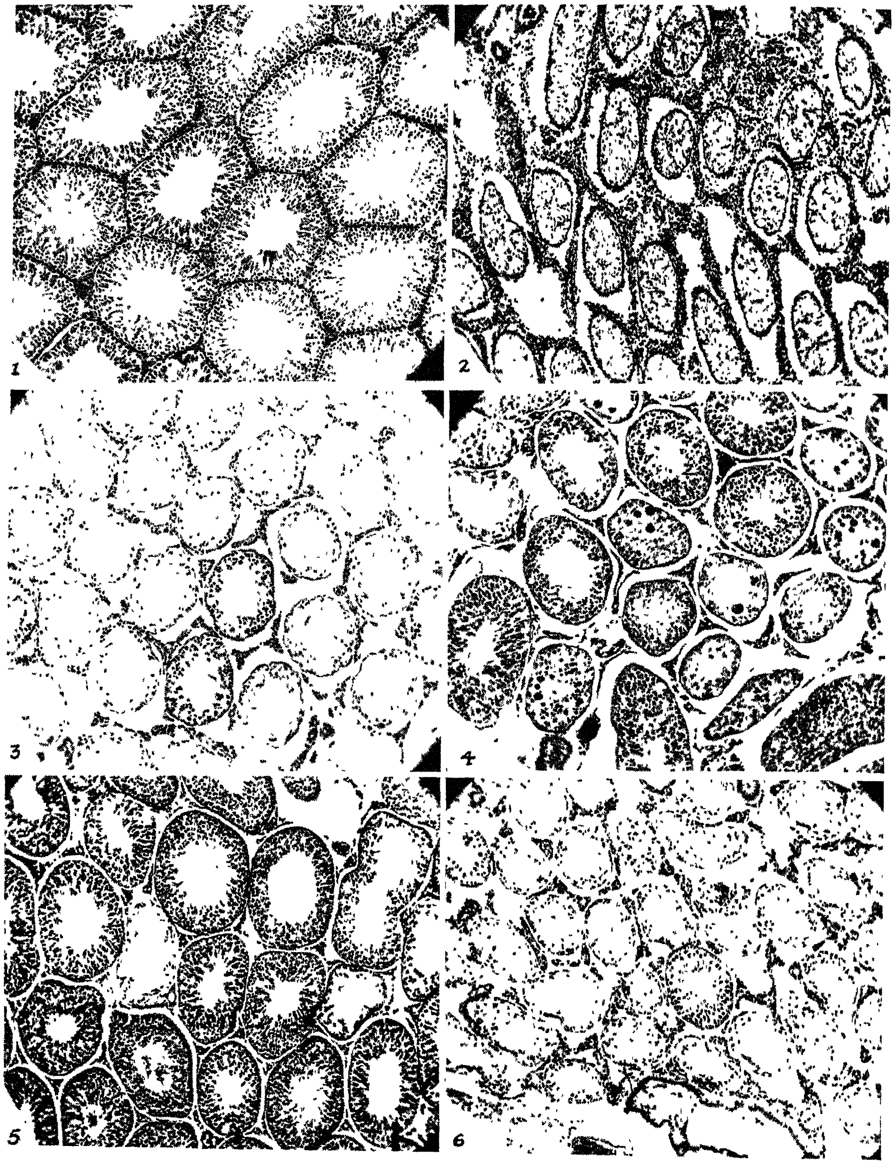

FIG. 1. Section from the normal testis of a male (M64) 43 weeks old. He was fed a good stock ration throughout. Acknowledgement is made to Dr. A. R. Lamb who supplied this section of a normal testis.

FIG. 2. Section from the right testis of No. 1263 (See Table I). Reduction in the size of the tubules and absence of germinal epithelium may be noted. Some of the shrinkage is probably due to the method of fixation.

[Legend continued on opposite page.]

complete idea as to the actual reduction in amount of tissue because of the very marked edema present. In some cases it was impossible to weigh the testis because in trimming off the epididymis the capsule of the testis was accidentally snipped and fluid oozed out leaving the testis almost flat. This edema may be noted in some of the figures presented.

With regard to the histological findings, our observations are not of sufficientvariety to state what are the steps in the process of degeneration.* In most of the testes examined microscopically the end result is all that was apparent. This state is characterized by the complete disappearance of the germinal epithelium leaving only a loose syncytium of the sustentacular cells (Sertoli cells). The tubules are greatly reduced in size and are often flattened and misshapen. In very advanced cases the number of tubules is also much reduced and edema, in some cases very pronounced, is always present (See Figs. 1—6).

In a few of the testes of the first generation animals examined, the degeneration was not quite complete and in some of the tubules multinucleated giant cells were seen. In the excellent description of the testicular degeneration due to low vitamin E published by Mason (2) a similar "giant cell" stage has been described. The final disappearance of the germinal epithelium leaving only Sertoli cells is also a characteristic of low E male sterility. However, Mason did not apparently encounter the very marked reduction in the size of the tubules and the pronounced edema observed by us.

Discussion

The testicular degeneration which we have described above we believe to be unique in that it apparently is not caused by low vitamin E. In support of this view we would draw attention to our work presented in the immediately preceding paper showing that female rats on identical diets have

Fig. 3. Section from the right testis of No. 1325 (Table I). Two tubules still possess some vestige of germinal epithelium.

Fig. 4. Section from the right testis of No. 1426 (See Table I). So-called "giant" cells may be seen in many of the tubules.

Fig. 5. Section from the right testis of No. 1427 (See Table I). Two tubules possessing practically no seminiferous epithelium can be seen. The other tubules are apparently normal.

Fig. 6. Section from the right testis of No. 1449 (See Table I). One tubule still retains its germinal epithelium while the others are degenerate.

All of the above photomicrographs were taken at a magnification of about 90 diameters. The original photos were reduced about one-half for reproduction.

Grateful acknowledgment is made to Dr. T. H. Bast of the Department of Anatomy who made the photomicrographs.

produced living young. Many of these females were litter-mate sisters of the males described above who produced litters long after the males were completely sterile. Poor reproduction was actually observed in these females, but, as we have shown, the reproductive mechanism failed in a manner entirely different from that occasioned by low E. If the testicular degeneration is due to low vitamin E, then there must exist an enormous difference in susceptibility between the two sexes, a difference which has not so far been noted by other workers.

Another point of interest lies in the fact that the male sterility occurring on the milk, iron, and copper diet is apparently intensified by the presence of the ferric chloride which we used as a supplement. In earlier work we studied the reproduction of males and females confined to a whole milk diet supplemented only with copper. We encountered no evidence of male sterility on these diets until we discontinued the animals. Because we were then aware of the sterility occurring in males on the iron and copper supplemented diet, we examined the testes of several of the former males. In one male kept for 46 weeks on the milk supplemented with copper, histological section of the testis showed almost complete degeneration. The brother of this animal, caged with him for the same length of time on the same diet, showed a practically normal testis. In other males kept for from 34 to 40 weeks on a copper supplemented milk diet the epididymal contents showed normal numbers and excellent motility of sperm. In two males on a similar diet after 51 weeks the testes were small and the smear from the cut surface of the epididymis of one showed only a few non-motile sperm. The smear from the other rat's epididymis showed a less than normal number of sperm, but there was excellent motility.

These observations indicate that indeed the testis may eventually be affected on the copper supplemented milk diets but not to the degree that occurs when iron as ferric chloride is further added to this diet. In the observations recorded in this paper the sterility was complete generally between 30 and 40 weeks on the latter diet. What the nature of the change caused by the iron is we cannot yet decide. We have already reported (3) a marked effect of adding ferric chloride to dry rations.

With regard to the record of No. 1426, it may be that the explanation lies in a variation in resistance of different animals. That his brothers did show marked testicular degeneration and that within his own testis some degenerate tubules were seen, lends support to the belief that he may have been affected earlier and then recovered. The possibility of recovery from such an advanced sterility, if caused by lack of vitamin E, would be small

according to Evans and Burr (4, p. 39). The iron dosage received by this group (0.5 mg. Fe per animal daily) was undoubtedly higher in the early weeks than that received by others fed 1.0 mg. Fe per 100 cc. of milk, since young rats do not usually consume 50 cc. of milk daily. Later, however, his iron intake was less than that of animals in other groups because as the rats near maturity as much as 100 cc. of milk may be consumed. It must also be mentioned that just prior to the time No. 1427 was discontinued, our milk supply was changed. Whereas it had been produced by cows receiving wheat straw as their only roughage, it was now supplied by cows receiving alfalfa hay and silage as part of their ration. We do not know whether or not this had a modifying effect on the course of the sterility, because of insufficient data.

As to the possibility in general of recovery from this sterility by means of a change in the diet, we cannot say anything definite. This, together with many other details of the problem, remains to be worked out.

Summary

1. Total sterility in male rats has been observed on a diet of cow's whole milk supplemented with small amounts of iron and copper salts. This sterility is characterized by the complete disappearance of the germinal epithelium, great loss in amount of testicular tissue, and pronounced edema.

2. This sterility is not due apparently to lack of vitamin E.

3. The effect on the testis is greatly intensified by some action of the ferric chloride. What this action is, is unknown.

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VITAMIN E IN IRON TREATED DRY RATIONS*

By

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IN 1928 we reported (1) the occurrence of sterility in female rats on mixed diets that had been treated with an ether solution of ferric chloride. The sterility was characterized by resorption of fetuses and was cured by adding daily doses of wheat germ oil to the diet of the affected females. We interpreted our results, therefore, as showing that the iron treatment had destroyed in some way the vitamin E *potency* of the diet. In this paper we wish to report further work which we undertook in an attempt to elucidate the manner in which the iron brings about this effect.

In addition to studying female performance, we have studied the effect of such a diet on the testes of males. The results secured from these experiments will also be reported below.

For a complete description of the ration which we used we would refer the reader to our earlier publication (1). It consisted of a mixture of 2/3 of a dry stock ration in use in this laboratory and 1/3 whole milk powder.[1] This mixture of stock ration and whole milk powder will be referred to throughout this paper simply as stock ration.

The iron treated ration was prepared by taking 99 per cent of stock ration and adding to it an ether solution of 1 per cent of ferric chloride. Sufficient ether was added to cover the ration in a flat dish. This was then set before a fan at room temperature and the mixture stirred at intervals until dry. Fresh batches of ration were made up every week or oftener.

Male Sterility on the Iron Treated Ration

The work of Evans and Burr (2), Mattill and associates (3), and Mason (4) has shown that male rats become sterile when grown and maintained on diets lacking in vitamin E. The sterility is occasioned by a disturbance of

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† E. R. Squibb and Sons Fellow.

[1] Klim, Merrell-Soule Company, Syracuse, New York.

normal spermatogenesis, such that at first non-viable sperm are produced and that later pronounced degeneration of the seminiferous epithelium takes place.

To determine if the iron treated ration would bring about sterility in males, as it had done in females, we took young male rats at weaning and fed them this diet. One group of seven received the iron treated ration alone; a second group of seven received the iron treated ration and in addition 200 mgs. wheat germ oil each daily (6 times per week). The first group was kept in one large cage provided with a screen bottom while the latter group was caged individually in screen-bottomed cages so that the wheat germ oil, which was fed in a separate dish, could be accurately measured to each.

The animals of both groups grew very well. Fifteen weeks after being started, the males of the first group averaged over 325 gm. while the males of the second averaged over 350 gm. Part of this difference was caused by an individual in the former which had an apparent infection of the respiratory tract and which weighed 262 gm. at the end of the 15 weeks. This individual also suffered from an everted penis (priapism?) which showed evident inflammation and scarring and hence he was discarded. His left testis weighed (trimmed) 0.33 gm.

After about 20 weeks on these diets the males were all placed in individual cages so that test matings with young normal females could be made. The females were of proven fertility and were placed with the males in their cages when in the early stages of estrus.

The mating performance of all the 13 males was very poor. A total of 44 opportunities to mate was given the six males remaining on the iron treated ration and only 9 positive matings were noted. The 7 males on the iron treated ration plus wheat germ oil had 54 opportunities to mate and 14 positive matings were recorded. One male of the first group and two of the second group never copulated, even though as many as 10 and 12 opportunities were afforded each of them. We were very much surprised at these results because it has been emphasized by Evans and Burr (2) that sex interest is maintained in males for very long periods even long after the fertilizing power has been lost.

From the nine positive matings by the males of the first group, no pregnancies resulted. From the 14 positive matings by males of the second group only 3 pregnancies resulted. This again was surprising since we had expected that, even though sterility would supervene in the males of the first group, those of the second group would be protected by the wheat

germ oil. An explanation doubtlessly lies in the fact that the oil which we used was one of low potency even though the dosage was relatively high.

Following these test matings all of the animals, except three in each group, were discontinued after approximately 26 weeks on the diet. The right testis from each animal was saved for histological study.

The three males from each group that were retained were placed in cages along with normal females and all were fed the stock ration. Here a true difference showed up between the two groups of males. From the females, caged with those that had received wheat germ oil, litters were secured at frequent intervals while no pregnancies occurred in the females caged with the males which had received only the iron treated ration. Later the males were exchanged between the two groups of females and again litters were born in the group which contained the males fed wheat germ oil while the other females produced no young.

These six males were later discarded and the right testes saved for histological study.

Sections from the testes of all the animals that had received only the iron treated ration showed complete or almost complete absence of germinal epithelium. Even in those three that had been maintained later for many weeks on the untreated stock ration, no histological evidence of recovery was noted. Marked edema was also noted in these testes. Six of the seven testes from the group which received wheat germ oil were sectioned and examined and all displayed an apparently normal structure.

It was apparent from these results that the iron treated diet was active in bringing about sterility in males. Those receiving this ration only were shown to be sterile after 20 weeks and those discontinued after 26 weeks were found to have lost practically all of the germinal epithelium from their testes. Even in those three that had been changed to the stock ration no evidence of testicular recovery was noted. It was, in fact, noted that their testes were flabby and edematous when they were discontinued. The trimmed left testis from one weighed only 0.73 gm.; that from the second weighed 0.90 gm.; while the testis from the third weighed 0.76 gm. Damage had been wrought beyond repair.

In contrast the testes from the group receiving wheat germ oil were histologically normal. The explanation of the poor fertility in this group during the test matings is probably the fact that the dosage of wheat germ oil was conferring only minimum protection. Evans and Burr (2, p. 39) have stated that there is a period of early infertility in males on low vitamin E in which the power to fertilize eggs is lost but that no structural

change may be noted in the testis. It is probable, therefore, that such a stage was reached in the males of this group when the test matings were first made. Those discontinued showed no histological evidence of degeneration while those changed to the stock diet completely recovered.

We cannot explain the lowered sex interest and activity exhibited by these two groups of males.

Further Experiments on Iron-Treated Diets

In our earlier work on female sterility brought about by iron treated rations, we observed a total absence of initial fertility. This point was of much interest in view of the fact that initial fertility, due to the reserves of vitamin E with which the animal is born, has been observed by Evans and his coworkers on nearly all rations studied by them. As a means of testing the iron treated ration further, and also to prepare sterile females for further experiment, we set about to raise many young females on this diet.

During the course of a month 94 females were taken at weaning and placed on the iron treated ration. Groups, containing for the most part 7 individuals, were kept in large cages fitted with screen bottoms. After 12 or 14 weeks, when the animals were well grown, stock males were placed in their cages so that mating might take place. At the same time vaginal smears were made daily on the females of seven of these groups so that accurate information could be obtained on their reproductive performance. Animals in which positive mating and implantation sign were noted, were weighed daily so that all criteria for the establishment of resorption were observed. Our experience was a repetition of what we observed earlier, *viz.*, that sterility was present in all females even in the first gestation. No young were born to any of these females, all the fetuses being resorbed. Those groups on which no vaginal smears were made were observed frequently to determine if any young were born, but none was found.

These observations, and many others which we made subsequently, permit us to state that females raised on the iron treated ration, prepared as we have described above, are invariably sterile by the time they reach maturity.

That the iron concentration of the ration was not of itself responsible for the sterile condition in the female rats, was indicated in our first work. There it was shown, and we have substantiated it in later work, that sterile females continued on the same diet but given in addition wheat germ oil (fed separately) produced young in the next succeeding gestation. It was

apparent that the effect was due to a change which the iron brought about in some constituent of the diet—the most likely of which was the fat. To determine if the iron would bring about the same result in the ration when added as a watery solution, and particularly where no drying was permitted, we performed the following experiment.

Sterile females from the groups discussed above were mated again and on the day of positive mating were segregated and fed the stock ration treated with ferric chloride dissolved in water. The same iron concentration was used as before but sufficient water was added so that the mixture after stirring was quite fluid in the feed cup. A fresh preparation was fed each morning, any food which remained from the day before being discarded. This method was followed throughout the period of gestation with the results indicated in Table I. There it may be noted that the females in which pregnancy was established bore living young and that the sterility

TABLE I

PERFORMANCE OF STERILE FEMALES ON STOCK RATION TREATED WITH AQUEOUS SOLUTION OF FERRIC CHLORIDE

Animal number	Diet	Date of mating	Placental sign	Young born
996	Stock ration treated	1/20		Ovulates, 11 day cycle
1058	with water solution	1/20	2/2	9 young
	of ferric chloride.			
1102		1/22		Ovulates, 11 day cycle
	Fed fluid daily.			
1057		1/24		Ovulates, 12 day cycle
	No drying.			
1090		2/5	2/18	7 young
1092		2/7	2/20	5 young (3 dead)
1080		2/13	2/26	6 young

was overcome. It was apparent, therefore, that drying with the accompanying aeration was in all probability a necessary factor in the destruction or inactivation of the vitamin E.

Other experiments performed later throw further light on this phase of the problem. As a means of determining if the iron treated ration could be prepared in a less expensive way by using less ether or else by using water as a solvent, we raised three groups of females from weaning to maturity on the following modifications of the ration. One group received the ration prepared by adding all the iron to 1/10 of the ration by means of ether solution. After this was dried before a fan it was intimately mixed with the untreated nine-tenths. The second group was fed a ration prepared

exactly as above except that the iron was added to the one-tenth of the ration by means of water solution. The third group received a ration prepared by adding the iron in water solution to the whole amount of the ration. The two rations which received the iron in water solution were finely ground after the drying process was complete. All three rations had the same iron concentration as the ration described above, *viz.*, 1 per cent of ferric chloride, and all were made up every week or oftener.

The three groups, each consisting of seven females, were mated after about 10 weeks on these diets. Vaginal smears were made daily on all the females.

Young were delivered by a majority of these females at the end of the first gestation, only a very few showing resorption. Second gestation performance, likewise, indicated a high degree of fertility although more resorptions were encountered than in the first gestation.

These results suggested that the sterility-producing effect of the original iron treated ration was evidently brought about by intimate contact of the iron and the ether-soluble material in the ration. This again directed our attention to the fats.

In addition to its effect on the vitamin E of a mixed ration, we have also found that iron will inactivate more concentrated sources of vitamin E. For example, we have treated wheat germ with an ether solution of ferric chloride and noted a very marked destruction or inactivation of the vitamin. Whereas the untreated wheat germ in daily doses of 250 mg. was found to bring about fertility in sterile females, raised and maintained on the iron treated ration, amounts four times as great (1.0 gm. daily) of the treated wheat germ did not prevent resorption of fetuses. (See Table II.)

Samples of wheat germ oil were also rendered partially impotent by long-continued aeration at 100° C. in the presence of iron. Fifty cc. of wheat germ oil were mixed with 100 cc. of ether containing 1 gm. of ferric chloride in solution. This mixture was placed in an oven at approximately 100° C. and heated air was bubbled through it for 10 hours. The ether soon evaporated leaving the iron distributed throughout the oil. Such treatment brought about very pronounced discoloration and the formation of some tarry residue which settled out. The aerated oil was poured through filter paper before being used. When fed at a level of 500 mg. daily during a gestation to sterile females which had been raised and kept on the iron treated ration, it did not prevent two of three females which became pregnant from resorbing again. (Table II.) The fact that one of the females produced young indicated that destruction or inactivation was not com-

plete. It may be noted that Evans and Burr (5) reported no deterioration in wheat germ oil aerated at 100° C. for 10 hours. The very marked inactivation in the wheat germ suggests that the increase in surface aids the reaction brought about by the iron.

TABLE II
THE EFFECT OF IRON TREATMENT ON WHEAT GERM AND WHEAT GERM OIL

Female number	Dietary change	Date of mating	Placental sign	Young born	Remarks
1097	250 mgs. wheat germ daily during gestation, fed separately.	1/15	1/29	8	5 dead.
1099		1/18	2/1	8	
1055		1/20	2/2	3	1 dead. Female died in parturition. 5 more young found in utero. Mature but dead.
1101		1/22			Ovulates, 11 day cycle.
1049		2/11	2/24	6	
1095		2/20	3/5	7	
1093	1 gm. iron treated wheat germ daily during gestation, fed separately.	2/28	3/13	0	Resorption.
1006		3/1			Ovulates, 12 day cycle.
1110		3/2	3/15	0	Resorption
1116		3/5	3/17	0	Resorption
1113		3/11	3/24	0	Resorption
1105		3/12	3/25	0	Resorption
1098	500 mg. wheat germ oil daily during gestation, fed separately.	2/5	2/17	10	
1075		2/7	2/20	5	
1076		2/8	2/21	?	Female dropped 41 gm. in wt. Small amt. blood noted around vulva. Young consumed?
1107		2/11	2/25	2	
1005	500 mg. iron treated wheat germ oil daily during gestation, fed separately.	2/21			Ovulates, 12 day cycle.
1111		2/21	3/6	6	
1096		2/22	3/8	0	Resorption
1084		2/23	3/6	0	Resorption
1106		3/7			Ovulates, 12 day cycle.

IS THE STERILITY PRODUCED BY THE IRON-TREATED RATION DUE TO THE PRESENCE OF AN "ANTIVITAMIN"?

In 1927 Evans and Burr (6) noted that certain fats and fatty acids (lard, hydrogenated vegetable fat,[2] oleic acid) when mixed with small doses of

[2] Crisco.

wheat germ rendered it impotent in the cure of sterility. They postulated the presence of antivitamin substances as an explanation (5). Their work, in conjunction with the experiments which we have described above, suggested to us the possibility that the sterility produced by the iron-treated ration might be due not so much to an actual destruction of vitamin E as to the production of some substance (or substances) which prevents vitamin E from acting. If such a substance was produced in the ration we thought that it might be possible to extract it. If it was extracted, the residue remaining might be able to support fertility. To test this point we performed the following experiments.

Two hundred fifty gram portions of the iron treated ration were extracted with ether at room temperature. The extraction was carried out by pouring several portions of ether over the ration in a medium-sized percolation funnel, the bottom of which was plugged with a wad of cotton. The dried residue, remaining after the extraction, was fed to sterile females beginning with the day of positive mating of the second gestation. This residue was fed *ad libitum* throughout the gestation to five females with the results indicated in Table III. Three of the females resorbed while two produced living young. These results, although not conclusive, suggested that, at least, the ration had been improved by the extraction and that the

TABLE III

THE EXTRACTION OF THE ANTIVITAMIN FROM THE IRON TREATED RATION BY MEANS OF ETHER

Female number	Diet	Date of mating	Placental sign	Young born	Remarks
1065	Females fed the ether	2/25	3/7	0	Resorption
1115	extracted residue from	2/25	3/10	0	Resorption
1003	iron treated ration *ad*	2/27	3/12	4	
1089	*libitum.*	2/27	3/12	9	
1103		3/6	3/18	0	Resorption
1122	230 gm. of stock ration	4/10	4/23	7	
1121	treated with iron-free	4/11			Ovulates, 12 day cycle.
1117	ether extract from 250	4/15	4/28	0	Resorption
1118	gm. of iron treated ra-	4/23	5/6	0	Resorption
1124	tion.	5/20	6/2	0	Resorption
1123	Iron treated ration, 250	4/10			Ovulates, 13 day cycle.
1126	mg. daily wheat germ	4/12			Ovulates, 13 day cycle.
1125	treated with iron-free	4/16	4/29	0	Resorption
1120	ether extract from 15	4/25	5/9	0	Resorption
1119	gm. of iron treated ra- tion.	4/27	5/10	0	Resorption

ether extract might contain some of the antivitamin. We decided to test this by seeing if it would induce sterility when added to rations known to be amply supplied with vitamin E.

The reddish brown ether extract was found to contain large quantities of iron. This we decided must be removed so that no further iron effect could take place when it was added to other rations. The extract was, therefore, washed with several portions of 10 per cent HCl, since it was found that washing with water would not remove the iron. The acid washing was followed by two washings with water. The extract so treated was found to be practically iron free.

Sterile females raised and held on the iron treated ration were mated again and on the day of positive mating were segregated and fed the untreated stock ration to which had been added this iron free ether extract of the iron treated ration. Because the extract from 250 gm. of iron treated ration carried approximately 20 gm. of fat, we added this to 230 gm. of the stock ration. This was dried and stirred before a fan at room temperature and fed to the sterile females throughout the second gestation. The results, summarized in Table III, show that three out of four females that became pregnant resorbed again, indicating that the ether extract did carry some fertility-preventing substance.

In a further experiment bearing on the same point, other sterile females were fed during a second gestation a daily dose of wheat germ to which was added the washed extract from 15 gm. of iron treated ration. To 250 mg. of wheat germ in a small mortar was added the measured quantity of ether extract. This was placed before a fan until all the ether was dissipated. The resulting mixture of fats and wheat germ was offered daily to mated females receiving the iron treated ration. Some difficulty was encountered in getting the females to consume this addition but by removing the other food for a short time and in some few cases by mixing in a small amount of the dry ration, consumption was secured. The results, also presented in Table III, show that three females resorbed.

These findings indicated to us rather convincingly that one of the effects of the iron treatment on the stock ration was the production of an ether soluble substance which prevented the vitamin E of the stock ration or of the wheat germ from acting.

The Effects of the "Antivitamin" on the Vitamin E Reserves of the Animal

The results from the experiment described above, in which the dried residue from the ether extraction was fed to sterile females, were subject

to more than one interpretation. The fertility observed in two of the females might have been due to small amounts of vitamin E remaining in the extracted ration, or it might have been due to greater reserves in the animals' bodies, these reserves being unable to act in the presence of the antivitamin. To gain further knowledge of the effect of the antivitamin on the vitamin E stores of the body, we devised the following experiment.

A group of seven young females were placed at weaning on the stock ration to which had been added 5 per cent of wheat germ oil. They were allowed to remain on this high vitamin E diet for two weeks, so that their bodily stores might be increased. They were then fed the iron treated ration. After 8 weeks on the latter ration, a vigorous male was placed in their cage and daily vaginal smears were started. In the course of about a month all of the females mated and completed a gestation. All showed typical resorptions. Following this they were changed to a basal ration[3] and allowed to continue their reproductive performance for several gestations. In the second gestation all the females, except one, resorbed again. One female bore a litter of 4 young, one of which was dead. In the third gestation five of the seven females produced young and living litters were born to some females even in the fourth gestation. In the fifth gestation all the females that became pregnant showed resorption. We present in Table IV these results.

In another group of seven females this same performance was observed. These females received the iron treated ration for a period of 9 weeks after weaning and were then changed to the basal ration. After about 4 weeks on this basal ration, a male was placed in their cage and daily vaginal smears were made on the females. They, too, were permitted to go through several gestations with no change in diet. Their records are shown in Table IV (Nos. 1305 to 1311). There it may be seen that three of them resorbed in the first gestation but that two of these three produced living young in the second gestation before going on to resorb in the third gestation performance.

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[3] The basal ration had the following composition:

Casein (alcohol extracted and heated)	18
Dextrinized Starch	69
Cod liver oil	2
Salts 40	4
Yeast	7

This diet contains no known source of vitamin E. We have also shown it to be unable to support fertility by rearing young female rats to maturity and having them resorb during either the first or second gestation.

TABLE IV
THE INEFFECTIVENESS OF HIGH STORAGE RESERVES OF VITAMIN E IN THE PRESENCE OF THE ANTIVITAMIN SUBSTANCE

Female number	Diet	First gestation	Dietary change	Second gestation	Third gestation	Fourth gestation	Fifth gestation
1267	Stock ration, 95%	4/16 resorption		5/27 resorption	6/26 resorption	8/29 resorption	
1268	W. G. O., 5%	4/14 resorption		5/19 4 young (1 dead)	6/22 9 young	7/21 4 young	9/12 resorption
1269	*Fed 2 weeks*	4/12 resorption	Basal ration	5/19 resorption	(Mated but did not become pregnant)		
1270	Iron-treated ration after 2 weeks	4/12 resorption		5/21 resorption	7/28 4 young (1 partly consumed)	9/12 resorption	10/26 resorption
1271		4/18 resorption		5/24 resorption	6/28 4 young (partially consumed)	7/27 5 young	8/23 resorption
1272		4/24 resorption		5/30 resorption	7/1 8 young	8/5 resorption	9/6 resorption
1273		4/11 resorption		6/2 resorption	6/29 11 young (1 dead)	7/25 6 young	
1305	Iron-treated ration fed 9 weeks	6/18 9 young		7/19 12 young	8/17 resorption		
1306		6/6 resorption		7/29 8 young	10/14 resorption		
1307	Basal Ration after 9 weeks	6/17 9 young (all dead) female died					
1308		6/12 resorption		7/22 5 young	8/18 resorption		
1309		6/30 9 young		7/28 4 young	8/25 resorption		
1310		7/6 8 young (1 dead)		8/2 resorption	9/7 resorption		
1311		6/25 resorption		7/23 resorption	8/20 resorption		

These results, we considered, were of extreme significance. They clearly indicated that the antivitamin of the iron treated ration effectively inhibited the action of the vitamin E of the body. That these bodily reserves were high, especially in the first group, was shown by the later fertility, even after subsisting for some time on a basal diet known to be low in vitamin E. The results with the second group were entirely similar, except that their reserves were not so high, since they did not have the high vitamin E diet at the beginning. Whether or not the iron treatment *destroyed* the vitamin E of the ration, it apparently did not destroy the vitamin E reserves of the animal.

These results also indicated that the antivitamin was either stored in the body for some time or so affected the reproductive mechanism that it could not respond immediately after the removal of the antivitamin. This interpretation was supported by the fact that in the first of the above groups, nearly all of the females resorbed in the first succeeding gestation after being changed to the basal diet. Two of the females of the second group resorbed in the first gestation even when they had been on the basal diet for more than 4 weeks. As stated before, these two females subsequently produced living young.

In addition to the above considerations, it was apparent that resorption could not longer be considered proof of the depletion of vitamin E stores in the animal body.

Discussion

In this paper we have presented further data on the sterility-producing properties of a stock ration treated with an ether solution of ferric chloride. We have shown that males as well as females become sterile when confined for a sufficient length of time on such a ration and that such male sterility is absolute. Wheat germ oil conferred protection, at least to the extent of preventing any testicular degeneration, which was histologically demonstrable. The lack of fertility in the mating records of the group that received wheat germ oil we can explain only on the assumption that incipient sterility, such as Evans and Burr (2) describe in their stage A, resulted because of the rather impotent oil that we used. So far as our studies go, the male sterility which we observed was similar to that occurring on low E diets.

In further observations made on many females raised to maturity on this diet, we have found that sterility was always present and that no initial fertility was possible. This female sterility, we have been able to show, is due, in part at least, to the production of some substance (or substances)

that effectively prevents the action of vitamin E. For lack of a better name we have followed Evans and Burr's suggestion in calling it an "antivitamin." This material may be extracted from the ration by means of ether and can be used to counteract the vitamin E of other substances.

The question has arisen in our minds as to whether the iron brings about destruction of E in the ration as well as the production of the antivitamin. Thus, for example, does the iron treatment when applied to wheat germ destroy the E known to be present as well as build up the antivitamin? This question we are at the moment unable to answer.

The production of such a high concentration of the antivitamin in the iron treated ration makes one wonder if this substance is entirely absent from synthetic rations. Synthetic rations contain iron salts as part of their salt mixtures, and fats in some concentration are nearly always present. Our results with the iron treated rations, in which the iron was added to a part of the diet by means of ether or water solution, and in which the whole ration was treated with watery ferric chloride, suggest that to obtain the greatest production of the antivitamin, intimate contact between iron and lipoid material is necessary. It is probable that in basal rations as they are ordinarily mixed, such intimate contact does not take place, and that the antivitamin effect is of small moment. However, the possibility of such an effect taking place under conditions which we have described should not be overlooked.

It has been shown in the foregoing pages that the antivitamin does not apparently destroy the vitamin E of the body. It does, however, effectively prevent its action so that animals with large body stores of vitamin E show uniform sterility on the iron treated ration. It is of interest to note also that the antivitamin effect does not disappear immediately when animals are changed from the iron treated ration to a basal ration. Its effect has been noted for more than a month after the change in the diet. Whether this is due to a storage of the antivitamin in the tissues of the body or to some effect on the reproductive organs which prevents them from functioning normally, we cannot decide. It may be stated, however, that we have kept females on the iron treated diet for over a year and subsequently had them produce living young when changed to the untreated stock ration. It appears, therefore, that no permanent injury to the organs of reproduction is wrought by long continuance on the iron treated ration.

With regard to the effect of the iron treated ration on phases of the reproductive function, other than that involved in the death and resorption of fetuses, we cannot speak definitely. We have encountered poor ovula-

tory rhythm in many females raised on this diet. We have, however, also noted the same phenomenon on many females raised and maintained on basal rations. We have been unable to explain this on the basis of lack of vitamin B since both iron treated and basal rations contained what we considered to be ample amounts. Since Evans has emphasized the fact that in low E all other phases of the reproductive function are normal we are apparently denied the explanation that these disturbances are due to a lack of E in such a diet. Therefore, it may be that the presence of the antivitamin affects other parts of the female reproductive apparatus as well as its proved effect in interfering with the nutrition of the fetus.

Summary

Additional experiments carried out on an iron treated stock ration have shown that this ration also produces sterility in male rats reared on it.

In further work with female rats we have found that this ration induces 100 per cent sterility. There is no first litter fertility.

We have shown that the iron treatment brings about the formation of a substance which actively opposes vitamin E. This substance may be extracted from the iron treated ration by ether. It has been shown that even in the presence of high storage reserves of vitamin E, females are unable to utilize these reserves in the presence of the antivitamin. After being changed to a basal ration, these storage reserves may become effective after 4 to 6 weeks. It has been suggested that the antivitamin is stored for that time in the tissues or else brings about some change in the reproductive apparatus of the female that prevents it from nourishing implanted fetuses.

The significance of these findings is briefly discussed.

Addendum

Since the above manuscript was written, there have appeared several excellent papers by Mattill and associates in which they have carried forward their earlier work, (Jour. Amer. Med. Assoc. LXXXIX, 1505, 1927), on the phenomenon of auto-oxidation of fats and its relation to the destruction of Vitamins A and E. In a recent article (this Journal III, 421, 1931) they have definitely correlated the susceptibility to oxidation of several fats and oils and the reproductive behavior of rats fed diets containing these fats. Their work shows that oxidation in the fatty constituents of the diet destroys vitamin E and that this oxidation is quite extensive in certain common fats and oils. It follows, therefore, that the amount of vita-

min E in any diet is markedly influenced by the balance between anti- and pro-oxygenic substances. It is very probable that what we have termed anti-vitamin is pro-oxygenic in character. Regardless of what name is applied, it is apparent that it has a profound effect not only in the ration, but also in the body of the rat.

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CYSTEINE AND TAURINE AS SUBSTITUENTS FOR CYSTINE IN NUTRITION

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THE intermediary reactions of the amino acids in animal metabolism have been investigated largely by means of a few simple methods, apparently susceptible to straight-forward interpretation. Among these methods may be mentioned, attempts to detect intermediary metabolites in the urine after flooding the tissues with the amino acid in question by administering excessive doses *per os* or parenterally; investigation of the fate in the body, mainly by examination of the urine, of suspected intermediary metabolites; investigation of the fate of amino acids and their derivatives when perfused through surviving organs, generally the liver; *in vitro* treatment of amino acids by mild oxidizing and reducing agents, or by intracellular enzymes obtained from tissue extraction; biochemical study of certain peculiar metabolic abnormalities involving disordered amino acid metabolism. As Dakin (1) has pointed out, however, the obvious interpretation of the results of these methods may not be the true one in so far as the elucidation of normal metabolism is concerned. In particular, "the behavior of a substance when gradually produced, at low concentration and rapidly undergoing further change, may be very different from that of the same substance when rapidly injected in relatively high concentration into the tissues of an animal. Indeed, in the latter case it is always uncertain whether the substance ever really reaches the sphere of action in the cells normally concerned with its metabolism." The dangers of drawing close analogies between chemical reactions produced *in vitro* and those occurring in the body are obvious, while "it is always difficult to decide how closely reactions observed under pathological or abnormal conditions resemble those occurring in the normal organism."

The current facts and theories concerning amino acid metabolism are based largely upon the results of these methods of study, and the divergent theories coexisting in the current literature of this chapter in biochemistry may often be traced to divergent interpretations of the same set of data,or

to an emphasis upon one set of data to the entire exclusion of another set possessing an apparently contradictory significance.

The advent of a new method of attacking at least some of the same old problems is, therefore, a hopeful sign and offers some promise of being able to discriminate among the available data on amino acid metabolism between those that relate to normal metabolism and those that relate only to the particular set of experimental conditions under which they were obtained. By this new method, rations deficient in some one amino acid indispensable for growth are supplemented with possible or probable metabolic derivatives of the amino acid in which they are lacking. If the ration thus supplemented will support a more rapid growth than originally, or if it will support growth when originally it would not, it is evident that the amino acid derivative producing this increased efficiency is serving the unique functions of the amino acid itself, probably by conversion into it. Hence if, from other considerations, it is a probable normal metabolite of the amino acid to which it is chemically related, the probability of this being true is appreciably increased.

By this method, Cox and Rose (2) and Harrow and Sherwin (3) have shown that 4-imidazole lactic acid is apparently a complete substitute for histidine in metabolism. The latter investigators obtained evidence that 4-imidazole pyruvic acid could also substitute for histidine. It may be inferred from these results that histidine may undergo either oxidative or hydrolytic deamination. Similarly Jackson (4) and Berg, Rose, and Marvel (5) proved that tryptophane-deficient diets would promote growth when supplemented with indole-pyruvic acid.

Provided that the basal diet has been proven deficient in growth-promoting power, and that this deficiency has been related entirely to the amino acid in question, the significance of positive results obtained by this method is clear. However, the significance of negative results, in which supplements have failed to give evidence of improving the growth-promoting value of the basal diets, cannot be so definitely assessed. The supplement may depress the food intake of the experimental animals or be otherwise toxic to them, and yet be a normal metabolite and even under other conditions a successful substituent for the related amino acid. According to Dakin (1) it is "perfectly possible for substances of high toxicity to be constantly produced and rapidly transformed into other bodies, so that the relative concentration of the toxic substance is always low." Or the intake of food, both on the basal diet and on the supplemented diet, may be so low that a possible superiority of the supplemented diet cannot

be demonstrated except by an exact equalization of the intake of food of test and control animals, a refinement of the method that has not hitherto been attempted. The fact that Harrow and Sherwin obtained positive results with imidazole-pyruvic acid, which had yielded only negative results for Cox and Rose, and that Jackson and Berg, Rose, and Marvel obtained positive results with indole-pyruvic acid, which had yielded only negative results for Heft and Sherwin (6) constitutes objective evidence of the inconclusiveness of failures to obtain obvious responses to dietary supplements in this method of study.

Even if these possible interpretations of negative responses to dietary supplements in the method under consideration could be disposed of, the inability of the amino acid derivative to evolve a positive response in increased growth is still of uncertain significance, since it may be due, either to the fact that the derivative is not producible in metabolism from the amino acid to which it is chemically related, or that it is produced normally from the amino acid but by reactions which are all or in part irreversible, either under all conditions, or under the particular experimental conditions chosen, including the other chemical constituents with which the amino acid derivative is fed.

With respect to cystine, all attempts at substitution thus far recorded, except those involving dipeptides of cystine (7), have given negative results. Westerman and Rose (8) were unable to secure evidence of a successful substitution for cystine of dithiodiglycollic acid, β-dithiodipropionic acid, or α-dihydroxy-β-dithiodipropionic acid (9). The substitution of taurine for cystine in nutrition was apparently successful in experiments reported by M. L. Mitchell (10), but later attempts to confirm this work, reported by Beard (11), Lewis and Lewis (12), and Rose and Huddlestun (13) were unsuccessful. Lewis and Lewis obtained similar negative results with cysteinic acid. In another publication (14) Lewis and Lewis fed elemental sulfur to young rats receiving two types of cystine-deficient diets. In the words of the authors, "no evidence was obtained to suggest that sulfur can replace cystine or in any way alter the cystine requirements for growth in the young white rat." Although diglycyl-cystine and dialanyl-cystine are able, according to Lewis and Lewis (7), to promote growth when added to cystine-deficient diets, no indications were obtained that the dianhydride of dialanyl-cystine could successfully substitute for cystine.

Experimental evidence of the type discussed at the beginning of this article furnishes support for the belief that the first step in the catabolism of

cystine is its reduction to cysteine, representing the conversion from a di-amino-dicarboxylic acid to a mercapto derivative of alanine. Lewis and McGinty (15), Lewis, Updegraff, and McGinty (16), and Rose, Shiple, and Sherwin (17) have shown that certain conjugated derivatives of cystine are in part excreted in the urine as the corresponding derivatives of cysteine, while the latter investigators have also shown that a conjugated cysteine derivative, phenyluraminocysteine, when injected into rabbits, is in part excreted in the urine as the corresponding cystine derivative. This agrees with other evidence in indicating that cystine and cysteine, as well as some of their derivatives, are interconvertible in the body, the conversion reactions being reversible.

In order to find out whether feeding experiments would confirm injection experiments in indicating a conversion of cysteine to cystine in metabolism the former was used as a supplement to a cystine-deficient diet in paired-feeding experiments with growing rats. Taurine was also tested in the same way, because the information on the value of this compound as a substituent of cystine in nutrition is divided.

Plan of Experiment

In both tests, the basal ration to which the supplements were added contained, as its main source of protein, dried skim milk powder to provide approximately 8 per cent of protein. According to numerous tests in this laboratory, such a ration when properly supplemented in other respects than protein is capable of supporting a fairly rapid rate of growth, but its growth-promoting value will always be increased when it is supplemented with cystine (18). The basal ration contained 20.41 per cent of dried skim milk, 4 per cent of the Osborne and Mendel salt mixture, 10 per cent of clear butterfat, 10 per cent of sucrose, 4 per cent of a cellulose flour[1] to furnish roughage, 1 per cent each of common salt, dried yeast (Northwestern Yeast Co.) and cod liver oil, and 48.59 per cent of starch. In the cysteine ration, 0.41 per cent of cysteine hydrochloride replaced an equal amount of starch, and in the taurine ration 0.50 per cent of taurine replaced an equal amount of starch.

The cysteine hydrochloride and the taurine used were obtained from the Eastman Kodak Company. The former contained 8.93 per cent of nitrogen as compared with the theoretical content, 8.89 per cent. The taurine contained 11.01 per cent of nitrogen as compared with the theoretical content,

[1] Cellu Flour, a product put out by the Chicago Dietetic Supply House, containing 37.8 per cent of crude fiber.

11.20 per cent. The cysteine hydrochloride was readily and completely soluble in cold water and in a saturated solution of sodium acetate. In the latter solution a heavy precipitate of cystine was produced by the addition of a drop or two of a dilute solution of hydrogen peroxide. It is evident that the cysteine hydrochloride was not contaminated with any appreciable traces of cystine.

In each of the two tests 8 pairs of rats were used; the rats in each pair were of the same sex, generally of the same litter, and were approximately the same in initial weight. They were kept in individual, cylindrical wire cages with raised bottoms. In the cysteine test, one rat of each pair received the unsupplemented basal diet and the other rat the same diet with a small amount of cysteine included. The food intake of each pair of rats was kept the same, and was so regulated that one or the other rat was kept close to the limit of its appetite. The food given to each rat was weighed daily on a fine chemical balance, and the daily allowance was increased regularly until one rat of the pair refused some of its allowance. In this event the daily allowance for both rats in the pair was reduced until the rat responsible for the reduction cleaned out his food dish. The allowance was then increased. The refused food was always left in the food dish, except at the end of an experimental week, when it was removed, weighed, and thrown out. In order to assure equal intakes of food for each week of the experiment, so that the weekly gains of pair mates would be directly comparable, increases in food allowances were not made on the last day of each experimental week. The taurine test was conducted in a similar manner.

Initial and final weights were the averages of three consecutive daily weights. Intermediate weights were also taken at the end of each experimental week. At the end of each test the rats were etherized and the body length, from anus to tip of nose, was measured, as an index of growth.

Experimental Results

The cysteine experiment was continued for seven weeks with the results shown in Table I. In each of the 8 pairs of rats, the rat receiving cysteine made the greater total gain, and attained the greater body length. The average difference in gain between pair mates is 18.1 grams favoring the cysteine rat. The standard deviation of the eight differences in total gain is 6.52 grams, the average being 2.78 times the standard deviation. For eight paired differences, according to the probability table of "Student," the odds are less than 1 in 10000 that chance alone would produce an average

result as great or greater than 2.78 times the standard deviation of the sample. The evidence may therefore be considered conclusive that cysteine will correct a cystine-deficient diet in the nutrition of growth. This means that it is convertible into cystine in the anabolic processes of growth. It may be inferred that this conversion takes place within the body rather than in the intestinal tract, since within the latter reducing rather than oxidizing conditions obtain.

In Table I, a nutritive index has been calculated according to the suggestion of Cowgill and Drabkin (19), by dividing the cube root of the body weight (grams) by the body length (centimeters). The larger this index becomes, the better the nutritive condition of the animal, as measured, for example, by its degree of fatness. In this experiment, the control rat possessed the greater nutritive index in 5 pairs, the cysteine rat in one pair, while in one pair, the mates were equal in this respect.

TABLE II

A COMPARISON OF THE WEEKLY GAINS OF PAIR MATES IN THE CYSTEINE EXPERIMENT

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	–	±
1	+	±	+	±	–	+	+	–	4	2	2
2	–	+	+	–	–	+	–	+	4	4	0
3	±	+	+	+	+	+	+	+	7	0	1
4	+	+	+	+	–	+	+	+	7	1	0
5	–	–	+	+	+	+	+	–	5	3	0
6	+	+	+	+	+	+	+	+	8	0	0
7	+	+	+	+	+	+	+	+	8	0	0
			Totals per pair						Totals for experiment		
+	4	5	7	5	4	7	6	5	43		
–	2	1	0	1	3	0	1	2		10	
±	1	1	0	1	0	0	0	0			3

Note: A + sign indicates a greater weekly gain by the cysteine rat.
A – sign indicates a greater weekly gain by the control rat.
A ± sign indicates equal weekly gains by pair mates.

In Table II, a comparison of the weekly gains of the pair mates in this experiment is made. For each pair and each week a plus sign (+) indicates a greater gain for the cysteine rat, a minus sign (–) a greater gain by the control rat, and a plus-or-minus sign (±) an equal gain by pair mates. The superiority of the cysteine-containing ration was evident from the third week to the seventh. The break during the fifth week in the accumulation of evidence favoring the cysteine ration was due to the fact that at this

TABLE I
THE EFFECT OF A CYSTEINE SUPPLEMENT TO A CYSTINE-DEFICIENT DIET ON ITS GROWTH-PROMOTING VALUE

	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Cysteine	Control	Cysteine	Control	Cysteine	Control	Cysteine
Final weight, gms.	135	143	128	141	132	160	144	158
Initial weight, gms.	52	50	53	52	50	52	40	41
Total gain, gms.	83	93	75	89	82	108	104	117
Final body length, cms.	17.9	18.5	17.8	18.6	18.3	18.8	18.1	19.2
Nutritive index	.287	.283	.283	.280	.278	.289	.290	.282
Total food, gms.	371	369	405	406	455	456	415	415
	Pair 5		Pair 6		Pair 7		Pair 8	
	Control	Cysteine	Control	Cysteine	Control	Cysteine	Control	Cysteine
Final weight, gms.	139	159	150	180	122	137	126	140
Initial weight, gms.	43	43	40	40	43	40	34	34
Total gain, gms.	96	116	110	140	79	97	92	106
Final body length, cms.	18.2	19.1	19.0	19.6	18.0	18.7	18.3	19.2
Nutritive index	.285	.284	.280	.288	.276	.276	.274	.270
Total food, gms.	363	366	481	481	417	413	414	414

time the cysteine ration used, newly made up, was found to contain considerably less than 8 per cent of crude protein, apparently due to an error in weighing out the ingredients. A new ration was immediately made up. In the entire experiment, 43 of the weekly comparisons favored the cysteine rat, only 10 favored the control rat, and 3 favored neither. This evidence is also clear cut.

The taurine experiment is typical of a negative outcome of a paired-feeding test, as the results in Table III clearly show. In only 2 of the 8 pairs of rats was the total gain of the taurine rat greater than that of its control, while the reverse was true in 6 pairs. The average difference between the total gains of pair mates was only 3 grams, favoring the control rat. It is so evident from inspection of the data that this difference possesses no statistical significance, that no analysis of it will be attempted. In body length and nutritive index, also the pair mates are not to be distinguished.

TABLE IV

A COMPARISON OF THE WEEKLY GAINS OF PAIR MATES IN THE TAURINE EXPERIMENT

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	−	±
1	+	+	±	−	+	±	+	+	5	1	2
2	−	+	−	−	+	+	±	−	3	4	1
3	−	−	−	−	−	−	−	−	0	8	0
4	+	+	±	−	−	+	±	−	3	3	2
5	+	−	−	+	−	−	−	+	3	5	0
6	+	+	−	−	−	−	+	−	3	5	0
7	−	+	±	+	−	+	−	±	3	3	2
	Totals per pair								Totals for experiment		
+	4	5	0	2	2	3	2	2	20		
−	3	2	4	5	5	3	3	4		29	
±	0	0	3	0	0	1	2	1			7

Note: A + sign indicates a greater gain by the taurine rat.
A − sign indicates a greater gain by the control rat.
A ± sign indicates equal gains by pair mates.

The comparison of the weekly gains of pair mates, as given in Table IV, is also clearly indicative of a negative outcome of the experiment. Of 56 possible comparisons, 20 favored the taurine rat, 29 the control and 7 were inconclusive.

As indicating the relative extent to which test and control rations would have been consumed if no restrictions had been placed upon food consump-

TABLE III

THE EFFECT OF A TAURINE SUPPLEMENT TO A CYSTINE-DEFICIENT DIET ON ITS GROWTH-PROMOTING VALUE

	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Taurine	Control	Taurine	Control	Taurine	Control	Taurine
Final weight, gms.	126	131	139	142	136	129	123	120
Initial weight, gms.	54	56	60	60	59	60	50	52
Total gain, gms.	72	75	79	82	77	69	73	68
Final body length, cms.	18.4	19.0	19.0	18.5	18.5	19.0	18.3	18.0
Nutritive index	.272	.267	.273	.282	.278	.266	.272	.274
Total food, gms.	409	409	437	435	434	434	430	429
	Pair 5		Pair 6		Pair 7		Par 8	
	Control	Taurine	Control	Taurine	Control	Taurine	Control	Taurine
Final weight, gms.	114	105	126	122	118	111	148	141
Initial weight, gms.	51	46	57	56	54	52	58	56
Total gain, gms.	63	59	69	66	64	59	90	85
Final body length, cms.	18.2	17.7	18.4	18.8	17.9	17.7	19.0	18.8
Nutritive index	.266	.267	.272	.264	.274	.272	.278	.277
Total food, gms.	367	367	451	451	402	402	475	477

tion, the number of times that test and control rats left food residues, thus causing a restriction in the amount of food offered to the pair, is significant. In the cysteine experiment, the control rats refused food 53 times and the cysteine rats 33 times. In the taurine experiment, the control rats refused food 42 times to 59 times for the taurine rats. It appears from these figures that the cysteine-supplemented diet was consumed somewhat more readily than the control diet, but on statistical grounds it may be doubted whether this is anything more than a fortuitous outcome.[2] In the taurine experiment it is even more evident that the taurine supplement did not significantly affect the avidity with which the basal cystine-deficient diet was consumed.

CONCLUSIONS

Cysteine added to a cystine-deficient diet will improve its growth-promoting value in animal nutrition. It is therefore convertible into cystine in anabolism.

No evidence was obtained indicating that taurine possesses this property.

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[2] In 86 refusals, the ideal outcome, if chance alone determined it, would be 43 favoring either control or cysteine rats. The actual outcome deviated from the ideal by 10 in each direction. The standard deviation of chance events of this type is equal to $\sqrt{0.5 \times 0.5 \times 86} = 4.6$. The deviation from the ideal outcome is thus only slightly more than twice the standard deviation and might therefore have been due entirely to fortuitous factors.

UTILIZATION BY NORMAL ADULT SUBJECTS OF THE CALCIUM AND PHOSPHORUS IN RAW MILK AND IN ICE CREAM*†

By

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INTRODUCTION

IT IS generally recognized that a freely chosen American diet may not furnish adequate amounts of calcium and phosphorus, although it may give ample protein and fuel value. Milk is a good source of both calcium and phosphorus. Some studies have involved a comparison of calcium and phosphorus assimilation from various forms of milk available for animal and human nutrition. In no experiment has ice cream supplied most of the calcium and phosphorus required, although ice cream which is manufactured from milk and its by-products is used to such an extent that it is thought of as a typical American food. Much attention has recently been given to the influence of ultra-violet light upon calcium and phosphorus metabolism. Experiments here described were planned to study the comparative values of ice cream and raw milk as sources of calcium and phosphorus, using an acid-forming diet, as the American dietary tends to be acid-forming on account of the wide use of cereal and meat products. The subjects were normal adults, half of them receiving ultra-violet irradiations during the experiment.

HISTORICAL

There has been much discussion in the literature concerning the availability of calcium and phosphorus from raw and heat-treated milks. This has been reviewed by Willard and Blunt (1), including the work of Washburn and Jones (2), Magee and Harvey (3), Daniels and Loughlin (4), and Daniels and Stearns (5). Kramer, Latzke and Shaw (6) also dealt with this problem. These investigations point to the superiority of raw, evaporated, condensed, and quickly boiled milks over pasteurized and dried milks.

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Investigations have been conducted to study the relationships between ultra-violet light and the metabolism of calcium and phosphorus. Bethke, Steenbock and Nelson (7) and Hart, Steenbock and Elvehjem (8) found that ultra-violet light influenced the storage of calcium and phosphorus and the equilibrium of these elements in the blood of growing and of mature animals. Very few experiments have been conducted with normal human subjects. Hart, Tourtellotte and Heyl (9) found that an adult male on a calcium-deficient, acid-producing diet showed no special tendency to retain calcium or phosphorus when irradiated daily for 20 days. On the other hand, Burton (10) found high calcium and phosphorus retention for boys who received some ultra-violet irradiation while on a diet high in these minerals.

Blunt and Cowan (11) have reviewed most thoroughly the literature dealing with ultra-violet light in nutrition as well as the effects of the acid-base balance of the diet upon mineral metabolism. Finding the work on this latter problem full of contradictions, they cited particularly the work of Shohl (12, 13) which indicated that when no sudden shifts in acid-base balance took place the neutral diet promoted best calcium and phosphorus utilization for rats and apparently for normal children.

Experimental

The subjects of this investigation were 10 normal college women, five graduate students, and five under-graduates. None of the subjects had been taking cod liver oil previous to the experiment, and no one of them had been treated with ultra-violet light. Ordinarily the subjects had been spending little time out of doors, and during the experimental period this was particularly true. Five of the subjects were irradiated daily for a period of five minutes at a distance of 30 inches from the arc. The source of ultra-violet light was a quartz mercury vapour uviarc treater lamp,[1] operated at 110 volts on alternating current. The amount of irradiation given was considered suitable for the purpose and quite safe for the subjects, none of whom reported burning although there were mild effects upon the skin.

The experimental period of 18 days, begun the middle of October, was divided into six three-day periods. Two of these periods did not involve the collection of urine and feces as they were used for the subjects to become adjusted to their diets. The periods were as follows:

[1] Type R. T. Sp. 100, manufactured by the Cooper-Hewitt Electrical Company.

Period I	Preliminary; raw milk	Period IV	Preliminary; ice cream
Period II	Raw milk	Period V	Ice cream
Period III	Raw milk	Period VI	Ice cream

The diet for this study was acid-forming which, according to the literature, might be expected to influence the assimilation of calcium and phosphorus. All subjects were provided with a uniform amount of protein, fat, calcium, phosphorus, and sodium chloride. The caloric value of the diet was varied for the different subjects by means of cornstarch and sugar, so that body weights remained practically constant throughout the experiment. The calcium and phosphorus contents of the diet were calculated to be close to the minimum requirements for equilibrium so that differences in utilization would be more apparent. As a comparison was to be made of the availability of the calcium from ice cream and from milk, these foods supplied a large percentage of the calcium in the diet. Foods were selected which could be purchased and kept in quantity, so that the basal diet for the periods might be kept as nearly constant as possible.

TABLE I

FOUNDATION FOOD INTAKE IN GRAMS PER PERIOD

Food	Milk periods	Ice cream periods
Milk, raw	780	—
Ice cream	—	675
Apple	330	330
Bread	470	470
Meat	240	240
Orange juice	150	150
Oleomargarine	252	252
Prunes	150	150
Rice	180	180
Sodium chloride	3	3
Daily calories	1979	1979
Daily protein	58.2	55.3

The foods selected for the fixed diet (Table I) were ground lean round of beef, white bread, polished white rice, raw apple, orange juice, prunes, and oleomargarine. The required amount of beef, purchased at the beginning of the experiment, was freed of all gristle and bone, ground, thoroughly mixed, and weighed into individual portions. These individual portions were wrapped in oiled paper and kept at a temperature below freezing so that they were perfectly preserved until used. Rice, prunes, apples, and oranges were purchased in quantities to meet the needs of the

entire experiment. The bread, made in a uniform way, was secured fresh for each period from a local bakery.

The variables of the diet were ice cream and raw milk, each supplying approximately the same amount of calcium to the diet. The milk, obtained daily from the college dairy, was the product of several farm herds. Dairy products from this same source were used in the manufacture of the ice cream made according to current commercial practice. A large mix was made from butter, sweetened condensed skimmed milk, plain skimmed milk, sugar and gelatine, and contained 12.37 per cent fat, 10.62 per cent milk solids not fat, 15.00 per cent sugar, 0.40 per cent gelatine and 38.39 per cent total solids. The mix was processed by pasteurizing at 145°F. for 30 minutes, homogenized at 2500 pounds pressure, cooled at once to 40°F. and aged for 48 hours at 40°F. prior to freezing.

The rice, stoned prunes, oleomargarine, white bread, and salt were weighed each time in amount to supply the period requirement. The orange juice, pared and cored apple, ice cream and milk were weighed daily. Each subject prepared her own meals in the unit kitchens of the home economics building. The milk was consumed at the noon meal and was not heated. The ice cream, on account of its bulk, had to be served at both the noon and evening meals. Only distilled water was used for drinking and cooking purposes.

Aliquot parts of the milk were preserved daily with formaldehyde for analysis. A sample was taken from the ice cream mix for analysis, as the supply was uniform throughout the experiment. The rice was ground in a ball mill and analyzed separately. The food composite was composed of one-third the weight of the individual food intake of orange juice, white bread, stoned prunes, ground beef, and apple for all the periods. The residue from the oleomargarine was found to be negligible and was not analyzed. Great care was taken to insure uniform sampling of food. The composite was dried to constant weight at 80°C. in an electric oven, pulverized, and stored in tightly covered containers.

Careful collections of all feces and urine were made for Periods II, III, V, and VI, carmine being used to mark the feces of the different periods. The urine was measured, specific gravity was taken, and samples were saved for analyses. The urine was preserved with toluene. The feces were dried at a low temperature to a constant weight, weighed, pulverized and saved for analyses.

Determinations of calcium in the food, feces, and urine were made by the McCrudden Method (14) from samples ashed in a muffle furnace. For

the phosphorus determinations the volumetric method given in the Official and Tentative Methods of Analysis by the Association of Official Agricultural Chemists (15) was used, magnesium nitrate being used in ashing.

The dietary calculations for calories and protein and the preliminary estimations of calcium and phosphorus contents of the diet were made from the tables of Rose (16). The food intake per period and the acid-base balance of this food as estimated from the tables of Sherman and Gettler (17) and Forbes (18) are indicated in Table I and Table II. The diet was acid-forming to the extent of 42 cc. of normal acid per period, without the prunes, for which no figure was available.

TABLE II

EXCESS OF ACID-FORMING OR BASE-FORMING ELEMENTS IN THE DIET PER PERIOD

Article of food	Grams of food	cc. of N acid or base per 100 grams		Total cc. acid	Total cc. base
		acid	base		
Apple	330		3.76		12.41
Bread	470	7.10		33.37	
Meat	240	13.91		33.38	
Raw milk	780		2.37		18.49
Orange juice	150		5.61		8.42
Oleomargarine	252				
Prunes	150				
Rice	180	8.10		14.58	
Ice cream	675				
Total				81.33	39.32

Note: The basicity of the ice cream was considered equivalent to that of the raw milk. Prunes also contribute to the acid-forming effect of the diet.

EXPERIMENTAL RESULTS

Table III gives the calcium and phosphorus content of the diet per period, as determined from analyses of all foods. The calcium and phosphorus contents of the raw milk and the ice cream and also the per cents of total calcium in the diet contributed by the latter two foods are given. The raw milk and ice cream furnished more than three-fourths of the calcium. The compositions of milk and ice cream are such that they necessarily furnished smaller proportions of the phosphorus, amounting to a little more than one-third of the total.

The figures for total intakes, output in feces and urine and the balances for calcium and phosphorus are given in Table IV. For comparison, balances were calculated for each period in grams per kilogram of body weight.

It is clear that the total intake of calcium was kept near the minimum required for maintenance in adults, as had been planned, so that differences might be evident.

For three of the irradiated and four of the non-irradiated subjects, the calcium balances for the ice cream periods were higher than for the fresh milk periods. Three subjects of each group showed better phosphorus balances for the ice cream periods.

Though well aware that averages from a small number of cases are not conclusive, it seemed of interest to compare the average calcium and phosphorus balances in grams per kilo per period, as follows:

Calcium			Phosphorus		
	Milk	Ice cream		Milk	Ice cream
Irradiated subjects......	−0.004	−0.005	Irradiated subjects.....	+0.011	+0.012
Non-irradiated subjects...	−0.003	−0.001	Non-irradiated subjects.	+0.018	+0.018

Each figure is the average for the five subjects in that group. For the non-irradiated subjects, the calcium of ice cream gave the more favorable balance, while for irradiated subjects, the calcium of fresh milk seemed slightly better. The non-irradiated subjects showed calcium balances at least as favorable as those of the irradiated subjects. The non-irradiated subjects also showed somewhat better phosphorus balances than the irradiated subjects, but practically no differences appeared between the milk and ice cream periods.

It is evident that the calcium from ice cream, containing condensed sweetened skim milk, was at least as well utilized as the calcium of raw milk, when furnished at a low level of calcium intake to normal adults on a diet somewhat acid-forming. More advantageous calcium utilization was not found in the group receiving ultra-violet irradiations, under conditions as described. As the compositions of milk and ice cream are such that they necessarily furnished smaller proportions of the total phosphorus in the diet, the results for phosphorus metabolism are less convincing although apparently similar to those for calcium.

Summary

A metabolism study was made to compare the utilization by 10 normal adult subjects of the calcium and phosphorus in raw milk and ice cream. The diet was somewhat acid-forming as is common throughout this country. Five of the subjects received ultra-violet irradiations daily.

TABLE III
CALCIUM AND PHOSPHORUS IN THE DIET

	I		II		III		IV		V		VI	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Grams for period	1.252	1.984	1.252	1.984	1.252	1.984	1.322	1.900	1.328	1.978	1.328	1.978
Grams from raw milk	0.984	0.792	0.984	0.792	0.984	0.792						
% from raw milk	78.59	39.92	78.59	39.92	78.59	39.92						
Grams from ice cream							1.060	0.766	1.060	0.766	1.060	0.766
% from ice cream							80.18	40.32	79.82	38.73	79.82	38.73

The raw milk contained 0.126 per cent calcium, and 0.099 per cent phosphorus.
The ice cream contained 0.157 per cent calcium, and 0.133 per cent phosphorus.

TABLE IV
CALCIUM AND PHOSPHORUS BALANCES IN GRAMS PER PERIOD (AVERAGES OF PERIODS USED)
SUBJECTS IRRADIATED

Subject, weight	Dairy product	Calcium				Phosphorus			
		Intake	Output	Balance	Balance per kg.	Intake	Output	Balance	Balance per kg.
L.D. 60.7 kilos	Milk	1.252	1.547	−0.295	−0.005	1.984	1.922	+0.062	+0.001
	Ice Cream	1.328	1.550	−0.222	−0.004	1.978	1.747	+0.231	+0.004
A.G. 49.3 kilos	Milk	1.252	1.448	−0.196	−0.004	1.984	1.312	+0.672	+0.014
	Ice Cream	1.328	1.414	−0.086	−0.002	1.978	1.235	+0.743	+0.015
I.G. 50.5 kilos	Milk	1.252	1.658	−0.406	−0.008	1.984	0.846	+1.138	+0.023
	Ice Cream	1.328	1.628	−0.300	−0.006	1.978	1.048	+0.930	+0.018
M.P. 60.9 kilos	Milk	1.252	1.324	−0.072	−0.001	1.984	1.811	+0.173	+0.003
	Ice Cream	1.328	1.644	−0.316	−0.005	1.978	1.450	+0.528	+0.009
L.R. 50.0 kilos	Milk	1.252	1.466	−0.214	−0.004	1.984	1.269	+0.715	+0.014
	Ice Cream	1.328	1.783	−0.455	−0.009	1.978	1.368	+0.610	+0.012

TABLE IV (continued)
SUBJECTS NOT IRRADIATED

Subject, weight	Dairy product	Calcium				Phosphorus			
		Intake	Output	Balance	Balance per kg.	Intake	Output	Balance	Balance per kg.
F.D. 55.7 kilos	Milk	1.252	1.381	−0.129	−0.002	1.984	0.894	+1.090	+0.020
	Ice Cream	1.328	1.154	+0.174	+0.003	1.978	1.129	+0.849	+0.015
E.H. 54.3 kilos	Milk	1.252	1.280	−0.028	−0.001	1.984	0.808	+1.176	+0.022
	Ice Cream	1.328	1.677	−0.349	−0.006	1.978	0.963	+1.015	+0.019
N.K. 72.9 kilos	Milk	1.252	1.610	−0.358	−0.005	1.984	1.140	+0.844	+0.012
	Ice Cream	1.328	1.542	−0.214	−0.003	1.978	0.906	+1.072	+0.015
N.S. 50.6 kilos	Milk	1.252	1.166	+0.086	+0.002	1.984	0.916	+1.068	+0.021
	Ice Cream	1.328	1.193	+0.135	+0.003	1.978	0.884	+1.094	+0.022
R.W. 50.9 kilos	Milk	1.252	1.792	−0.540	−0.011	1.984	1.264	+0.720	+0.014
	Ice Cream	1.328	1.503	−0.175	−0.003	1.978	0.990	+0.988	+0.019

No special advantages in the utilization of calcium and phosphorus seemed to be conferred upon those subjects who received ultra-violet irradiations, as described, throughout the 18 days of the experiment.

Four of the non-irradiated and three of the irradiated subjects showed more favorable calcium balances when ice cream was the chief source of calcium. Normal adult subjects on an acid-forming diet utilized the calcium from ice cream made with condensed milk at least as well as the calcium of raw milk. In general the phosphorus balances followed the trend of the calcium balance figures.

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VEGETABLES IN THE DIETS OF PRESCHOOL CHILDREN*

By

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VARIETY AND FREQUENCY OF VEGETABLES IN THE WEEKLY DIETARY

AMERICAN families eat more vegetables than they did ten years ago. The increased consumption is reflected in quantities shipped from the larger markets. For example, the consumption of three vegetables as indicated by shipments from eleven markets more than doubled in six years.[1]

		1920	1926
Cauliflower	Crates	2,343,000	5,550,000
Lettuce	Crates	7,928,000	17,236,000
Spinach	Tons	49,600	119,200

This change in the family diet together with the more marked attention of the past five years to the habits of the preschool child have affected his diet.

Before he cuts his teeth the child is given specially prepared vegetables. The demand for pulped and strained vegetables has been great enough to bring many commercial products on the market. At what age the young child may have any or all vegetables from the family table is still an unsettled question. That between three and five years he is getting a wide variety is made evident through surveys of typical groups. (See Table I)

The variety of vegetables and the frequency of serving them to preschool children are brought out in Table I which presents data gathered by keeping daily records of the food of 42 children for periods varying from one to four weeks as shown in Table II. As indicated in Table II, none of the summer and early fall months when fresh vegetables are most plentiful was included.

* This study was made possible through the co-operation of Professors Thomas Vance, Lulu Lancaster, and Lydia Swanson of the Department of Child Development.

[1] Figures from the U. S. Dept. Agr. Yearbook, 1926, p. 956.

TABLE I

AVERAGE NUMBER OF SERVINGS OF VEGETABLES PER CHILD PER WEEK

Group	I	II	III	IV	V	VI
Asparagus	.1	.0	.0	.2	.9	2.6
Beans, snap	1.0	1.8	2.0	1.7	1.9	.6
Beets	.3	.6	.3	.0	.6	.4
Cabbage	.6	1.3	.2	1.0	1.6	.7
Carrots	1.6	2.4	1.4	3.0	2.1	2.7
Cauliflower	.1	.2	.0	.0	.1	.5
Celery	.9	1.2	.6	1.0	1.2	.6
Corn	.2	.5	.0	.2	.3	.4
Lettuce	2.0	3.2	4.9	1.5	3.3	2.5
Onions	.0	.3	.1	.2	.4	.2
Parsnips	.2	.0	.0	.0	.1	.0
Peas	1.4	2.1	1.6	1.2	2.1	1.7
Spinach	.8	1.0	1.8	1.3	1.3	1.0
Squash	.1	.1	.0	.0	.0	.0
Sweet potatoes	.5	.4	.0	.0	.0	.0
Tomatoes	1.5	1.4	2.4	1.2	2.4	2.4
Turnips	.3	.0	.0	.5	.7	.0
Potatoes	6.1	6.9	7.4	6.7	—	—
Others and mixtures	1.5	.9	1.1	3.0	.9	.1
Total excluding potatoes	13.1	17.4	16.2	16.0	19.9	16.4

TABLE II

TIME OF SURVEYS AND CHILDREN INCLUDED

Group*	Season	Duration of survey wk.	Children		Age		
			Total no.	Girls no.	Average yr.-mo.	Min. yr.-mo.	Max. yr.-mo.
I	Nov.-Dec.	4	6	2	4- 5	3- 2	5-3
II	Nov.-Dec.	4	7	3	3- 8	2-11	4-8
III	Jan.-Feb.	1	13	7	4- 2	3- 3	5-0
IV	Jan.-Mar.	1	6	3	4- 1	3- 8	4-8
V	Apr.	2	5	3	4- 6	4- 0	5-0
VI	Apr.-May	2	5	2	3-11	3- 8	4-2

* The children of Groups I and VI did not attend a nursery school.

These children are receiving more than two servings of vegetables per day in addition to a serving of potatoes. Nine or more different vegetables are served per week to the average child without taking into account the

different ways of preparing them for the table. Lettuce, carrots, and tomatoes are most commonly used, with peas and snap beans next.

In 1911 Griffith (3) advised that carrots, parsnips, squash, stewed tomatoes and turnips be given with extreme caution to children of three to six years. He advised avoidance of cabbage, cauliflower, raw celery and raw tomatoes. Most of them are common foods in the child's diet now.

Nutritive Value of Vegetables in the Mixed Diet of Children

Because in many cases the servings of vegetables are very small, an individual dietary study of six nursery school children was carried out to determine what contribution vegetables make to the diet, especially in minerals.

The children chosen seemed representative of a large group though they may have fared better than the average child. They were in good physical condition as indicated in Table III. Their mental ratings by the nursery school psychologist were above the average for their ages. The parents were high school or college graduates, the fathers professional or business men and the mothers interested in feeding their children according to the best standards they knew.

Table III

Description of Subjects

	Sex	Age yr. mo.	Height in.	Weight lb.	Deviation from average* per cent
Teddy	Boy	3– 8	41.8	45.0	+18
Buster	Boy	3–10	39.0	34.0	−3
Reed	Boy	4– 5	43.6	44.8	+4
Bev. Ann	Girl	4– 5	41.4	42.0	+11
Persis	Girl	4– 6	41.5	38.2	0
Cornelia	Girl	4– 8	44.1	41.2	0

* Average weight as given by Woodbury.

Samples for analysis. All foods eaten by each child during a week were weighed and duplicate samples were collected. Vegetables, including potatoes, as free as possible from butter, cream or thickening, were kept separately. Milk samples were preserved with formaldehyde and kept in a refrigerator. All other foods were dried. Composite thoroughly mixed samples representative of a week's food for each child were secured for analysis. The water intakes for the week were measured.

Quantities of foods. Vegetables including potatoes, composed a significant portion of the diet. By weight as served they constituted one-seventh of the total mass of food, milk for drinking three-sevenths, and all other foods three-sevenths. The maximum quantity of vegetables as consumed by one boy was nearly three times the minimum eaten by the oldest girl. Their variety is shown in Table I, Group IV. The vegetables contained on the average 91 per cent water; other foods aside from milk contained 67 per cent water.

That the energy values of the diets of these children approximate the average for their age (5) is indicated in the last column of Table IV. The quantities of milk as shown in Column 3 appear small but these volumes do not include that incorporated in foods.

TABLE IV
AVERAGE FOOD CONSUMPTION PER CHILD PER DAY

	Vegetables as served	Servings of vegetables	Beverages		Total energy**
			Milk	Water	
	oz.	no.	cups*	cups*	Cal.
Teddy	6.8	2.4	1.7	0.6	1454
Buster	9.2	4.1	2.6	1.8	1577
Reed	8.2	3.8	3.2	1.0	1704
Beverly Ann	6.7	3.0	1.4	1.4	1429
Persis	5.1	2.4	1.8	1.9	1272
Cornelia	3.3	2.8	2.7	2.0	1235
Average	6.5	3.1	2.3	1.5	1445

* 237 cc.

** Calculated on basis that 145 gm. milk yield 100 Cal. (8) and one gram of a dried mixed diet yields 5 Cal. (2).

Methods of analysis. To determine the contribution of vegetables to the total minerals of the diet, all foods were analyzed as follows: for total ash by igniting samples in a muffle furnace at a temperature of about 400°C; for calcium by McCrudden's method (7), titrating the oxalate against standard permanganate; for phosphorus by Neumann's method (4), dissolving the phosphomolybdate in alkali and back titrating with standard acid; for iron by Kennedy's colorimetric method (6), extracting the sulphocyanate in isoamyl alcohol. Analytical data on water as reported by the State Experiment Station were used. For the iron content of milk, an average composition figure as reported by Sherman (8) was used.

Vegetables as a source of minerals. That the vegetables were comparatively high in minerals is shown by averaging the figures for the dried products for these six children (See Table V). The total solids of vegetables are higher in ash and in some individual minerals than is the dried product from the mixture of other solid foods in the child's diet. A liberal mineral intake may be secured by a liberal use of vegetables.

TABLE V

AVERAGE PERCENTAGE COMPOSITION OF DRIED FOODS

	Dried vegetables	Other foods, dried
Total ash	7.51	2.93
Calcium	0.21	0.16
Phosphorus	0.30	0.23
Iron	0.0073	0.0053

From the total food intake for the week and the percentage composition of the foods the average daily mineral intake of each child was calculated and is shown in Table VI. With the exception of the child who drank less than one and one-half cups of milk per day, the diets of these children were adequate in minerals according to commonly accepted standards.

TABLE VI

AVERAGE DAILY MINERAL INTAKE PER CHILD

	Total ash	Calcium	Phosphorus	Iron
	gr.	gr.	gr.	mg.
Teddy	9.50	0.98	1.00	13.3
Buster	10.58	1.20	1.26	14.3
Reed	11.83	1.47	1.48	13.8
Bev. Ann	7.74	0.85	0.86	13.1
Persis	8.82	0.91	0.98	16.9*
Cornelia	9.83	1.20	1.08	8.5
Average	9.72	1.10	1.13	13.3

* Liver eaten on 3 days of week.

The influence of milk on the total mineral intake is noticeable when the average percentages of the total food nutrients contributed by the vegetables in the diet are calculated and compared with those of milk (See Table VII). For calcium and phosphorus, the milk drunk is the principal

contributor and it furnished about four-tenths of the total minerals. The chief value of vegetables so far as these figures show lies in their comparatively high iron content. They furnished more than one-sixth of the total

TABLE VII

AVERAGE CONTRIBUTION OF FOODS TO THE MINERAL INTAKE

	Vegetables	Milk	Other foods
	per cent	per cent	per cent
Ash	2.3	42.1	54.7
Calcium	5.6	65.4	26.1
Phosphorus	8.3	52.2	39.5
Iron	17.3	9.8	72.4

iron intake. Just as the diet of the child receiving little milk was low in calcium and phosphorus, just so the diet of the child who ate only half as much vegetables as the average was far the lowest in iron. Hence, it is important that vegetables be used to a great extent in diets like those of the preschool child, which usually contain much milk.

Reasons for liberal use of vegetables. Besides contributing much iron and some other minerals, the vegetables furnished approximately 10 per cent of the total energy for these children and acted as a vehicle for butter and cream. The texture of the diet and its vitamin content, especially that of the antiscorbutic vitamin, are probably determined to a large extent by the vegetables.

But for another reason the vegetable content of the diet is important. As the child grows older, the quantity of milk in the diet remains stationary or even decreases while the consumption of other foods increases to meet the growing energy need. Among these foods the proportion of those rich in minerals and vitamins needs to be increased if the quantities necessary for growth and regulation of body processes are to be maintained. Perhaps nothing helps more in developing a liking for a particular food than does its early introduction into the diet. An appetite for a variety of vegetables may be developed during the preschool years, a transition period following infancy.

APPETITE OF PRESCHOOL CHILDREN FOR VEGETABLES

Much attention has been given during the past few years to the appetite of children because of many reports showing the prevalence of anorexia. Aldrich thinks that this lack of appetite is a late development for he says

(1) that the general practitioner of thirty years ago would be incredulous were he told that the biggest feeding problem today is to get the child to eat the foods he should. Physiological, hygienic or psychological factors may explain lack of appetite. Sometimes the influencing factor is dietary. We know little about the reasons for children reacting favorably to some foods and at the same time refusing to eat others equally desirable from the viewpoint of the adult. Several observers have said that vegetables are the least liked of all foods by children.

To get definite evidence of the attitude of young children toward vegetables, the nursery school at the noon hour presents the best opportunity because many children can be observed at the same time and under the same conditions.

Comparison of nursery school children and other preschool children. That the reactions of the nursery school children are representative of preschool children is supported by comparison of the ten children composing groups V and VI of our survey (p. 116.) They were observed at all their meals for two weeks; after the first meal the mothers recorded on mimeographed forms the responses of the children to vegetables as indicated by reactions to be designated later (p. 123). Comparison is made only between attitudes toward vegetables eaten at home. The nursery school children had a total of 145 servings of vegetables at home, the other group 164. In each group there was a definite liking for a vegetable manifested toward 77 per cent of the servings. The nursery school children liked 98 per cent of the raw vegetables served, the other group 85 per cent. The former group disliked a somewhat greater percentage of all the vegetables (10 versus 4 per cent) but there were included two children who refused to eat at home some vegetables they ate at school; one child announced that his mother's vegetables were black and those at school white. The order of eating vegetables among foods served was the same. The cooked products were not standardized and the judgments of mothers on the attitudes of their own children might be biased. However, this was no more likely to be true in one group than in the other. On the whole the two groups were very much alike in their responses.

For the main part of the appetite experiment, two workers observed six groups of nursery school children at their noon meal for periods five weeks long. The 55 children observed varied in age from 1 year 11 months to 4 years 10 months. Among these children it was an exceptional occurrence for a child not to appear to want to eat its lunch.

Of course conclusions reached after observing a small number of children

in a single community are tentative and can be regarded only as preliminary. Our results are recorded here in the hope that others may be interested in this aspect of nutrition. Though we may know just what foods should make up a child's diet, whether he eats certain ones in sufficient quantity appears to depend very much upon his attitude toward them.

Method of determining desire of average child. For the first two groups of 15 and 13 children, records were kept of all remarks concerning vegetables, of the order of eating vegetables relative to other foods served and also of the apparent desires of each child for the vegetables and for the meals as shown by his manner in eating them. To have a method of designating differences in desire for a food by various children and for various foods by a single child numbers were used as follows: 1 for great desire; 2 for a liking, 3 for indifference; 4 for a dislike; 5 for great repugnance. The same system was used to record the degree of apparent appetite of each child for his meal. It was thus possible to secure averages. An element of personal judgment enters in but two observers agreed well on any cases that were compared.

Two other groups of children were served, during their five-week period, twelve vegetables which were wholly new to them. So far as possible the methods of preparation of the vegetables were standardized. The service was regulated by giving very small portions and increasing them somewhat after the vegetable had been served twice. The position of the new vegetable on the plate was controlled by putting it at the child's left with the sandwich on the far side of the plate and the meat or egg near him. Records were kept of order of tasting and finishing the vegetable, of verbal remarks and of facial expressions that were inspired by vegetables. Remarks and facial expressions were classified into three types: interest or curiosity as "What is this?"; definite favor as "I like this" or "This is good"; definite displeasure as "This is nasty" or "I don't like this." No attempt was made to grade intensity of response by a child. Using the number of interest, favorable and unfavorable responses the vegetables were ranked and a total score for each secured; the procedure was repeated with facial expression indications.

Appetite as shown by manner of eating. The attitudes of the first group toward creamed turnips and toward a mixture of spinach and chard were indicated by an average figure 3 for each; they were eaten as a matter of course. Toward the other 11 common vegetables served a total of 22 ways there was noticeable desire. In general, the appetite of the second group for vegetables checked that of the first; they were not served creamed tur-

nips nor the spinach-chard mixture but their attitude towards creamed onions is shown by an average between 2.5 and 3.0, close to indifference. Except for cooked, strong-juiced ones, these 28 children liked common vegetables.

Appetite for vegetables as shown by remarks. While new vegetables were served 56 remarks of definite favor and 55 unfavorable remarks were overheard showing neither a great liking nor dislike for new vegetables. There were 228 verbal interest remarks made. Among these were 36 comparisons of the forms of the vegetables to familiar objects, as when broccoli flowerets were served, "My oak tree is all gone" or about French endive, "Is this celery?" Such remarks show interest among children in the appearance of their food. There were 10 interest remarks comparing the flavor of the vegetable to that of a familiar one. Among the vegetables fed both groups the final scores were not very different. Brussels sprouts and raw kohlrabi were liked best. No single property of a vegetable marked it for favor unless it were interesting in form or resemblance to some familiar object.

Appetite as shown by order of eating vegetables. Almost invariably the children ate their foods in rotation; in general when a food was tasted the whole serving was eaten before other foods were tasted. Cooked vegetables were usually eaten second or third with the bread and butter sandwich or the meat first. In the first group creamed turnips and in the second group creamed onions remained on the plate till last. In the groups fed unfamiliar vegetables, the order of tasting and finishing individual vegetables did not check in the two groups. Marked desire for a food seems to develop gradually.

Appetite for raw vegetables. In observation of the group of 10 children in their homes and again of the first groups in the nursery school the greater desire of children for raw vegetables was manifest. Nine common vegetables or vegetable mixtures were served raw to the first group and six to the second. Raw carrots and turnips cut in slender sticks were especially liked but cabbage shredded and served with dressing as a salad was received almost indifferently. After the first five-week period of feeding unfamiliar vegetables, those which had been most favorably received were fed during a second five-week period, making variations in texture, and this procedure was repeated with another group of children. Texture variations were made by serving the vegetable usually in four forms; raw sticks or large pieces, cooked sticks or large pieces, raw chopped and puréed cooked. Scoring variations in texture by tabulating verbal remarks showed no significant difference between the first three forms but the

puréed cooked were not eaten with relish. However, scoring by facial expression, an objective method, or by order of tasting and finishing the vegetable, the raw in large pieces was far in the lead if one accepts the assumption that young children eat first what they like best.

An experiment was carried out to test further this appetite of children for raw vegetables. During two five-week periods, two small groups, numbering seven and three children respectively, were allowed at three noon meals each week to choose between raw and cooked vegetables. In preparation of a vegetable, the edible portion was cut into uniform pieces, then half was crisped in ice water and the other half cooked until tender in just enough water to cover. Little plates with small bits of the raw and the cooked were placed on the table and the children were asked to taste both.

After the large plate with the rest of the food on it was put before the child he was allowed to choose the vegetable wanted. Out of 68 choices the raw was taken 55 times. Raw cabbage was taken 14 out of 15 times, raw carrots 18 in 22 times, raw cauliflower 7 in 12 times, raw celery 4 in 6 times and raw turnips 12 in 13 times. Whether due to more pleasing flavor or to crispness, raw vegetables were much better liked than cooked ones whether they were served in the form of sticks, cubes, large irregular pieces or were shredded or ground. In general, if they were cut so that they were readily picked up in the fingers they were eaten first, otherwise second or third among the foods served.

The principal objection to raw vegetables has been due to their fiber, a difficulty in masticating and an indication of indigestibility. The child seems to enjoy chewing crisp food. Perhaps we are passing through the period of caution with raw vegetables; many children are eating them with apparently great desire and without apparent harm, when care is taken to serve tender products only.

Summary

Records of the food of 42 preschool children show that they are eating a little more than two servings of vegetables per day in addition to potatoes. The variety has increased during the past twenty years to include most of the common vegetables.

An individual dietary study of six preschool children, with analysis of the food, showed that they receive 6 oz. of vegetables per day. This was one-seventh of the total weight of food as served and furnished about 10 per cent of the total energy and 17 per cent of the total iron. The total solids of mixed vegetables are rich in minerals.

It is important with preschool children to develop an appetite for vegetables so that an increasing quantity will be desired as the child grows. Feeding unfamiliar vegetables to 33 children certainly showed no refusal of new things introduced into the environment but rather the interest that young children have in new things and the strong appeal made by foods served in pieces of attractive shape. Repeated observations of 28 children showed that with the possible exception of strong-juiced vegetables, young children like vegetables to which they have become accustomed, especially if they are crisp in texture.

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THE VITAMIN B AND G REQUIREMENTS OF LACTATION*

By

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THE fact that an adequate quantity of vitamin B, old nomenclature, is in some way related to successful lactation has been observed for some time. Hartwell (1) in 1924 found that better milk production was possible in white rats if the amount of vitamin B in the diet was increased above the quantity necessary for growth. Guest, *et al* (2) in 1926 using cereal grains in the diet of white rats obtained results to show that the amount of vitamin B necessary for normal lactation is greater than that required for normal growth and that the amount of vitamin B required for reproduction is not much greater than that required for normal growth. The investigations of Sure (3) in 1927 showed that the young of mothers depleted of vitamin B developed paralysis, muscle chills, and screaming, running fits and that the symptoms were relieved by the administration of yeast. Macy and coworkers (4) in 1927 found that three to five times more vitamin B is necessary for lactation than for growth.

With the recognition of the two factors now believed to be a part of the original vitamin B, Evans and Burr (5) in 1927 reported that "the *additional* yeast needed for lactation is solely due to its addition to the antineuritic vitamin B and not to the growth-promoting vitamin B of the diet." Sure (6) in 1928 believed both factors to be necessary in added quantities for successful lactation. The present experiments have been carried on further to study the relative requirements of vitamins B and G for lactation. The data obtained are indicative of the fact that while both vitamins B and G must be present to obtain milk production, the lactation is usually more successful if vitamin G is fed in larger quantities than the amount which is given for growth.

EXPERIMENTAL PROCEDURE

Adult albino rats were used in these experiments. Each rat was placed in an individual, raised bottom cage. The food was placed in white por-

* The preliminary work of this investigation was submitted by Dorothy L. Hussemann in partial fulfillment for the degree of Master of Science in Home Economics in the Graduate School of the University of Illinois, 1929.

celain jars with screw tops, having an opening one and one-half inches in diameter. Supplements to the main diet were given in small, glass, castor cups. A few days previous to the birth of the young, the animals were transferred from the small cages to specially constructed cages, used in these experiments (Figure 1). No bedding was used until all of the young were born and then the rat was given shredded tissue paper for her nest. The cages, trays and food cups were changed weekly and the water cups, castor cups and bedding were changed daily. All of the apparatus was washed and sterilized with steam before being used again.

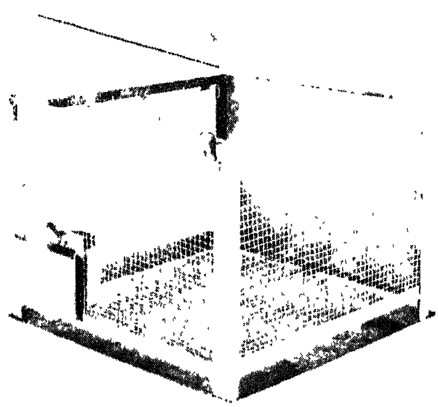

FIGURE 1.
The type of cage used in these experiments during the period of lactation. The animals were left in the small cages until just a few days before the young were born. At this time they were transferred to the large cage and allowed to remain there throughout the period of lactation.

The rats were weighed every three days until the birth of the young and then during the period of lactation were weighed every day. The litters, also, were weighed each day. The animals were mated in small cages, one male being used for each female. They were allowed to remain together for four days, at which time the male was removed. During the first experiments it was assumed that the female was pregnant and on the same day that the separation from the male occurred, she was transferred to the test diet. In the later experiments the female remained on the test diet throughout a preliminary rest period of three weeks in addition to the period of pregnancy and lactation. Four days after the birth of the young the litter was reduced to six (Evans and Burr, 5). Success in lactation was judged by

the growth of the young and their general nutritive condition. The period of lactation was considered to be twenty-one days in length.

The animals were allowed to eat *ad libitum*. The food intake was noted each time an animal was weighed or whenever a new supply of food was given. Distilled water was also given *ad libitum*. During the first experiments the female was placed on Diet A, called the "resting diet," for at least two weeks before mating. This was dispensed with, however, during the later experiments and the animals were given the test diet throughout the entire period. In addition to the "resting diet" Diets B, C, D, E, F, G and H were tested. The composition of the diets is given in Table I.

Observation will show the basal constituents of all of the diets to be the same, that is, they contain vitamin B-free casein[1] (except Diet A), dextrin or glucose,[2] Osborne-Mendel salt mixture, and various combinations of untreated and autoclaved yeast supplemented by cod-liver oil and wheat germ oil. The amount of casein in the diet was constant in all cases. It is approximately the same percentage as that used by Evans and Burr (5) in their studies on lactation. The amount of salt mixture incorporated into the diet is the same as that chosen by Chick and Roscoe (7) and Evans and Burr (5). Dextrin or glucose was a variable factor being increased or decreased according to the amount of yeast used. The dextrin was prepared by mixing cornstarch with cold water and then adding hot water. The mixture was placed in an oven at one hundred sixty-two degrees centigrade and allowed to cook until the mass in the pan was clear and opalescent, more water being added, if necessary. When the starch paste was clear the heat was reduced to one hundred and seven degrees centigrade and the oven door was opened slightly. In this manner the wet mass was allowed to dry. After being dried, the dextrin was ground to a fine powder and it was then ready for use as a source of carbohydrate in the diet. Because of the uncertainty as to the vitamin content of the dextrin, and the fact that in some growth experiments in our laboratory (10) glucose was shown to be free of vitamins B and G, glucose was used as a source of carbohydrate in the latter experiments. It was also found easier to use, requiring no preparation. Five drops of cod-liver oil were fed daily to each mature animal (Macy, 4). The wheat germ oil was made according to the cold acetone method of Sure (8). It was used to supply the vitamin E factor for which Evans and Burr (9) found the minimum protective dose to be 25 milligrams. This is approximately equivalent to three drops, so the latter amount was fed each day.

[1] Harris Laboratories, Tuckahoe, New York.

[2] Merck and Company, Rahway, New Jersey.

TABLE I

SHOWING COMPOSITION OF "RESTING DIET" AND OF TEST DIETS

RESTING DIET			
Diet A			Per cent
Casein—commercial			30
Salt mixture*			4
Untreated yeast			5
Dextrin			61
TEST DIETS	First Experiments	Later Experiments	
Diet B	Per cent		Per cent
Casein (Vitamin B-free)	30		30
Salt mixture*	4		4
Untreated yeast	15		20
Carbohydrates	51	dextrin	46 glucose
Diet C			
Casein (vitamin B-free)	30		
Salt mixture*	4		
Untreated yeast	5		
Autoclaved yeast	5		
Dextrin	56		
Diet D			
Casein (Vitamin B-free)	30		
Salt mixture*	4		
Untreated yeast	5		
Autoclaved yeast	10		
Dextrin	51		
Diet E		*Diet E*	
Casein (Vitamin B-free)	30		30
Salt mixture*	4		4
Untreated yeast	5		5
Autoclaved yeast	15		15
Carbohydrates	46	dextrin	46 glucose
Diet F		*Diet G*	
Casein (vitamin B-free)	30		30
Salt mixture*	4		4
Autoclaved yeast	15	untreated yeast	5
Dextrin	51		61 glucose
		Diet H	
		Diet G+3 drops of tikitiki daily	

* Osborne and Mendel.

In addition, in each case, five drops of cod-liver oil and three drops of wheat germ oil were given daily.

Tikitiki,[3] the dilute alcoholic extract of rice polishings, was used as a source of vitamin B. It has been shown by many workers to be potent in

[3] Bureau of Science, Manilla, Philippine Islands.

this factor. There is some indication, however (10), that tikitiki also contains vitamin G. The amount used in these experiments was the same as that used by Evans and Burr (5). An attempt was made to destroy the vitamin G fraction of yeast by irradiation following the technique of Hogan and Hunter (11) and then to use this product as a source of vitamin B in the diet. By testing the irradiated yeast on growth-experiment animals, however, it was found that there was practically no discernible destruction of vitamin G. This was later confirmed by Kennedy and Palmer (12) who found that only a very small quantity of vitamin G is destroyed by irradiation. Hence, in the experiments untreated yeast,[4] or untreated yeast and tikitiki were used to supply vitamin B in the diet. The source of vitamin G used throughout the experiment was autoclaved yeast. This was prepared by placing dried yeast in uncovered glass petri dishes to a depth of one-half inch. The yeast was not moistened and without further treatment was put in the autoclave. Here it was heated at one hundred twenty degrees centigrade with fifteen pounds of pressure for five hours. Then it was removed from the autoclave, dried, and ground to a powder. Frequent tests on growth animals showed this yeast to be a potent source of vitamin G, free from vitamin B.

During the first weeks of these experiments the yeast was given separately as a supplement to the diet. Such a procedure was unsuccessful as the animal often refused the yeast. The yeast was then incorporated into the diet in a definite percentage. Five per cent was the amount found by Evans and Burr (5) to be necessary for maintenance and growth and fifteen per cent was needed for lactation so these percentages were used in our control diets. In Diet B, however, the amount of yeast was raised to twenty per cent to test the effect of this higher level. The amounts and combinations in the other diets were chosen and set up arbitrarily.

Discussion of Results

Reference to Table II shows that only three of the eight litters born to mothers whose diet included 15 per cent yeast for vitamins B and G and dextrin for carbohydrate were successfully nourished during the period of lactation. Evans and Burr (5) have reported that "only when 15 per cent yeast is added to Diet 232 that normality in mortality and weaning weight is approached." Our results indicated that even more yeast might be necessary. In the later experiments, therefore, the yeast was increased to

[4] Northwestern Yeast Company, Chicago, Illinois.

TABLE II

SHOWING THE DIET, SOURCES OF VITAMINS B AND G, SUCCESS IN LACTATION, WEIGHT AND CONDITION OF YOUNG, AND CONDITION OF MOTHER AT THE END OF LACTATION

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young	Condition at the end of the Experiment	
					Gms.	Young	Mother
1493	G	5 per cent yeast	6	6	26.6	Small, slightly oily	Normal
801	B (First experiments)	15 per cent yeast	6	6	37	Nice	Normal
949			6	6	38	Nice	Normal
M25			6	6	32	Nice	Normal
793			5	None			
806			10	None			
807			14	None			
948			13	None			
948			10	None			
7	B (Later experiments)	20 per cent yeast	6	6	28.3	Sticky, yellow, oily	Sticky, yellow
1397			5	4	37.5	Nice	Normal
5			6	6	34.3	Fur not cleaned, diarrhea	Yellow and oily
1503			6	6	50.0	Nice	Normal
1502			6	6	46.3	Nice	Normal
1721			6	5	36.6	Nice	Normal
1722			3	None			

TABLE II (continued)

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young Gms.	Condition at the end of the experiment: Young	Condition at the end of the experiment: Mother
790	C	5 per cent untreated yeast and	10	None			
802		5 per cent autoclaved yeast	9	None			
951	D	5 per cent untreated yeast and	6	6	31	Fur thin	Normal
952		10 per cent autoclaved yeast.	6	6	26	Nice	Normal
M15	E (First experiments)	5 per cent untreated yeast and 15 perccent autoclaved yeast	6	2	16	Fur rough, gray paralysis and recovery	Normal
M18			6	5	34	Nice	Normal
949			6	6	34	Nice	Normal
1563	E	5 per cent untreated yeast and	6	5	30	Nice, small	Normal
1562	(Later	15 per cent autoclaved yeast	6	6	38.8	Nice	Normal
1500	experiments)		6	6	41.6	Nice	Mite sores
1498			6	6	38.6	Nice	Normal
1504			6	6	30.6	Nice	Normal
1727			5	None			
805, 806, 953	F	15 per cent autoclaved yeast	Mother became yellow and oily. Hemorrhages occurred at the time of parturition and the young were never born.				

TABLE II (continued)

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young	Condition at the end of the experiment	
					Gms.	Young	Mother
1570	H	5 per cent untreated yeast and 3 drops of tikitiki daily	6	6	38.3	Nice	Rough and slightly oily
1568			6	6	39.3	Nice	Normal
1569			6	6	38.0	Fur not clean	Abscess on mother's neck
1725			6	6	36.3	Nice	Normal
1724			10	None			
1459			1	None			

20 per cent and only one of seven litters failed. This would indicate that yeast at higher levels than 15 per cent is even more conducive to successful lactation. Evans and Burr (5) further found that yeast at a level of five per cent in the diet did not furnish enough vitamin B for lactation but this quantity was sufficient to maintain an adult rat in good health. Only one rat in our experiments was fed a diet containing five per cent yeast. It was found that she successfully nursed a litter of six for twenty-one days. The weaning weights, however, were low. The fact that this rat was able to successfully maintain the young may be due to a storage within her body of one or more factors from previous experimental work. Additions to the maintenance level of yeast of one or the other fractions of the vitamin B complex gave results indicating the relative importance of the two factors for successful lactation.

As has been stated, autoclaved yeast was used as a source of vitamin G. In Diet C five per cent autoclaved yeast was added to five per cent untreated yeast in the basal diet, the dextrin being decreased to this amount. Implantation occurred and the pregnancy was normal but when the young

were born very little if any milk was secreted and the young died within a week. Following the indication of so marked a deficiency in the diet, the amount of autoclaved yeast was doubled. At the level of 10 per cent autoclaved yeast and five per cent untreated yeast, two rats successfully nursed litters of six for twenty-one days but the weaning weights were low. A weight of 40 grams at the end of twenty-one days was the standard which had been adopted. A diet containing 15 per cent of autoclaved yeast with five per cent untreated yeast was then tested. The young obtained weighed from 16 to 38 grams at three weeks of age and were normal in appearance with the exception of one litter of two which showed a paralysis of the legs with subsequent recovery. On 15 per cent autoclaved yeast alone a successful litter was never born. At the normal time of parturition bleeding from the vagina occurred in the mother. An autopsy showed that the young had apparently developed normally at first but had become hemorrhagic.

Evans and Burr (5) have reported that "the additional yeast needed for lactation is solely due to its addition to the antineuritic vitamin B of the diet, for when tikitiki is given to lactating mothers without increased yeast dosage, we can also produce normal lactation." In the experiments here reported we have been able to repeat this work, four litters in six being successful on a diet which contained five per cent yeast and three drops of tikitiki. The weaning weights approached normal ranging from a weight of 36 to 39 grams at twenty-one days of age.

Hence, it would seem that if a source of either vitamin B or G is added to the maintenance level of yeast in the diet, more successful lactation occurs. The basal diets used for these animals were carefully controlled and tests showed these diets to be free of both vitamins B and G. That autoclaved yeast is a pure source of vitamin G we have shown in growth experiments. That tikitiki is a pure source of vitamin B has never been so definitely shown. Chart I demonstrates the results of growth experiments using tikitiki as the source of both vitamins B and G. It will be noted that almost normal growth was obtained when the larger amounts of tikitiki were used. This indicates that some vitamin G is present in tikitiki. The results obtained with tikitiki, therefore, are doubtful as to interpretation. It may be either vitamin B or vitamin G or even both of these vitamins which are functioning.

Chart II shows the growth curves of the young and mothers of typical animals on Diets B, E, H and G. There is very little difference in the condition of the mother and the young whether the source of the B and G vitamins is untreated yeast to 20 per cent of the diet or five per cent un-

treated yeast and 15 per cent autoclaved yeast. It would appear possible, therefore, to obtain successful lactation without increasing the intake of vitamin B over the maintenance level provided sufficient vitamin G is given. The young of mothers whose diet included five per cent untreated yeast and 15 per cent autoclaved yeast were in better general nutritive condition than those of mothers whose diet included five per cent untreated yeast and three drops of tikitiki.

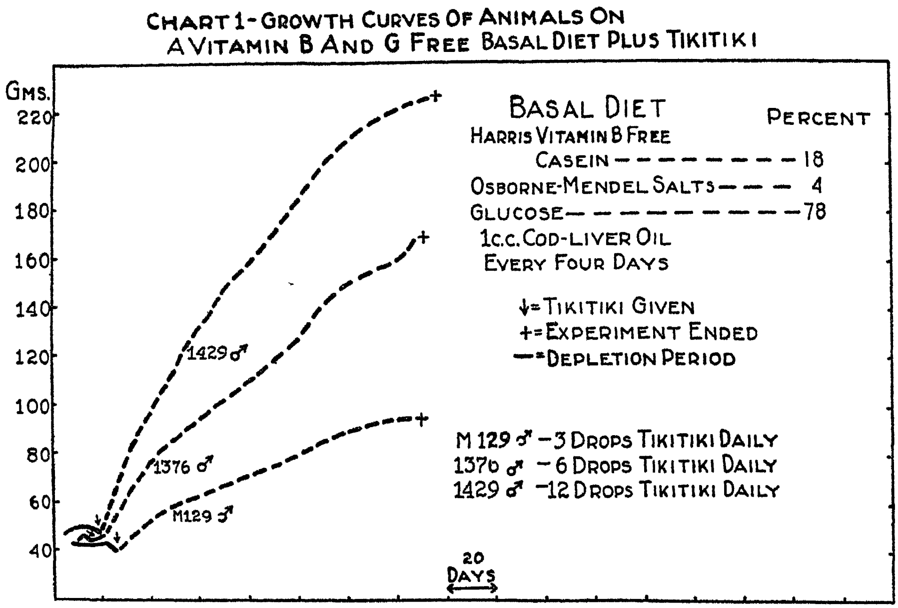

CHART 1.—Growth curves of animals on a vitamin B- and G- free basal diet plus tikitiki. The almost normal growth of animals receiving 12 drops tikitiki daily as the sole source of vitamins B and G indicates the presence of appreciable amounts of vitamin G in tikitiki.

Reference to Table III will show that in practically every case of successful lactation, there is a corresponding rise in food intake. All of the mothers who were able to suckle their young for twenty-one days ate comparable amounts of food during the period.

Summary and Conclusions

In this paper experiments are recorded which were devised to study the relationship of each factor of the vitamin B complex to lactation. Albino rats were used as experimental animals. Untreated yeast at a level of five per cent of the diet was used as a source of the vitamin B complex. This is

CHART 2-WEIGHT CURVES AND FOOD INTAKE OF TYPICAL ANIMALS ON DIETS B, E, H AND G

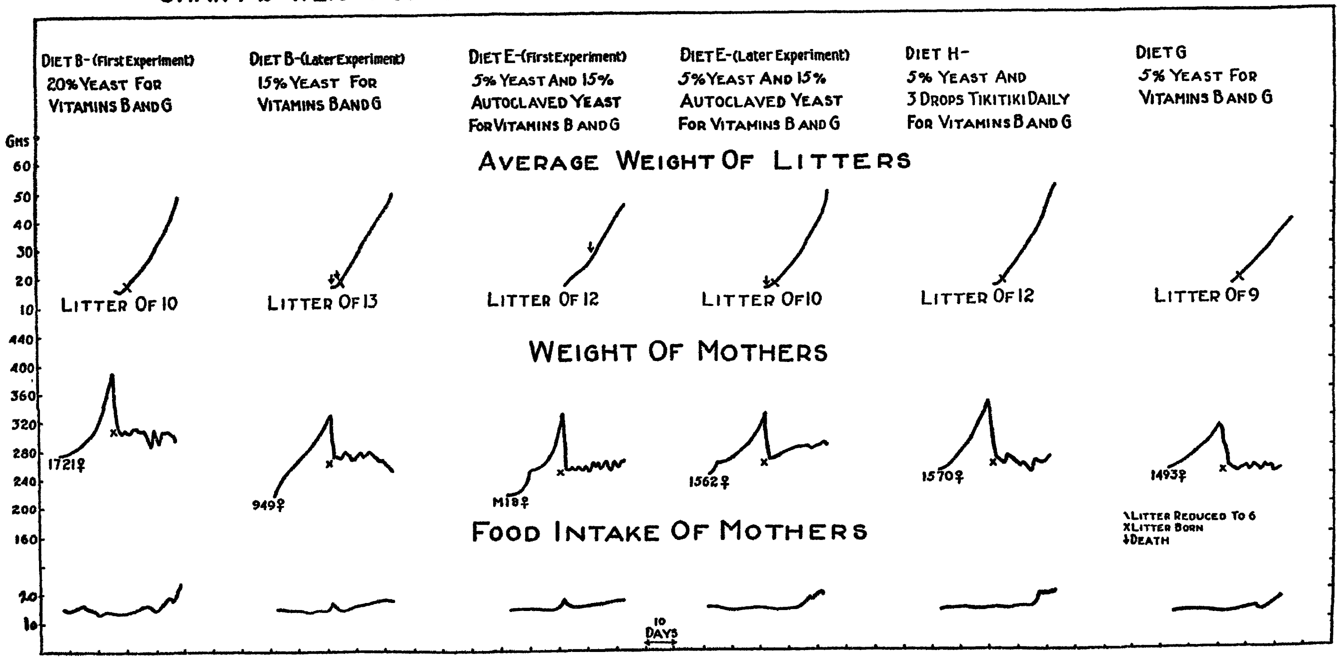

CHART 2.—Weight curves and food intake of typical animals on diets B, E, H, and G. These diets include varying amounts of vitamins B and G. The food intake and weights of the mother rats are given during the periods of pregnancy and lactation. The growth of the young is recorded throughout the lactation period and in every case is indicative of the adequacy of the mother's diet for successful lactation. The almost normal growth of young of mother rats receiving five per cent yeast and 15 per cent autoclaved yeast for vitamins B and G in contrast to the slow growth of young of mother rats receiving five per cent yeast as the sole source of these vitamins indicates that it is possible to obtain successful lactation without increasing the intake of vitamin B over the maintenance level provided sufficient vitamin G is given.

Note: Diet B (First Experiment) above should read 15% yeast, and Diet B (Later Experiments) should read 20% yeast.

known to furnish the maintenance level of these vitamins but is not sufficient for lactation. Either autoclaved yeast as a source of vitamin G or tikitiki as a source of vitamin B was given in added quantities and observations were made as to the effects on lactation.

Following this procedure these conclusions may be drawn:

1. In order to produce successful lactation in the rat, both vitamins B and G must be present in the diet. It may be that there is a definite quantitative relationship which exists between the two vitamins.

2. Either vitamin B or G in quantities increased above and added to

TABLE III

SHOWING THE DIET, SOURCES OF VITAMINS B AND G IN THE DIET, AND DAILY FOOD INTAKE OF EACH RAT BEFORE AND DURING PREGNANCY AND DURING LACTATION

Rat number	Diet	Source of vitamins B and G in the diet	Av. Daily food consumption before pregnancy (gms.)	Av. Daily food consumption during pregnancy (gms.)	Av. Daily food consumption during lactation (gms.)
1493	G	5 per cent yeast	12	16	19
801	B	15 per cent yeast	15	16	30
949	(First ex-		11	16	21
M25	periments)		10	20	20
793			12	11	7*
806			13	13	Refused food
807			14	9	6*
948			11	16	7*
948			11	15	12*
7	B	20 per cent yeast	14	19	26
1397	(Later ex-		10	17	39
5	periments)		12	14	26
1503			15	21	32
1502			13	18	24
1721			11	19	19
1722			12	17	Failed*
790	C	5 per cent untreated yeast	11	14	Failed*
		and			
802		5 per cent autoclaved yeast	9	17	Failed*
951	D	5 per cent untreated yeast	13	14	22
		and			
952		10 per cent autoclaved yeast	12	14	23

TABLE III (*continued*)

Rat number	Diet	Source of vitamins B and G the diet	Av. daily food consumption before pregnancy (gms.)	Av. daily food consumption during pregnancy (gms.)	Av. daily food consumption during lactation (gms.)
949	E	5 per cent untreated yeast	11	14	23
	(First experiments)	and			
M15		15 per cent autoclaved yeast	12	22	16*
M18			12	17	27
1563	E	5 per cent untreated yeast	Record lost	17	21
	(Later experiments)	and			
1562		15 per cent autoclaved yeast	21	17	34
1500			15	20	25
1498			14	18	27
1504			15	14	26
1727			14	22	Failed*
801	F	15 per cent autoclaved yeast	15	9	Failed*
806			13	6	Failed*
953			12	4	Failed*
1570	H	5 per cent untreated yeast	14	21	29
		and			
1568		3 drops of tikitiki daily	16	18	27
1569			15	20	26
1725			12	14	22
1724			13	14	Failed*
1459			13	18	Failed*

* These mothers were unsuccessful in lactation.

the maintenance level of the vitamin B complex yields more successful lactation. In view of the fact that just as successful results followed the use of autoclaved yeast, a source of vitamin G free from vitamin B, as were obtained when tikitiki, a source of vitamin B containing also vitamin G, was used in addition to the maintenance level of yeast, it seems possible that the added vitamin G is the more important for successful lactation.

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Editorial Review

A COMPARISON OF FEEDING STANDARDS FOR DAIRY COWS, WITH ESPECIAL REFERENCE TO ENERGY REQUIREMENTS*

OF supreme importance in relation to the nutrition of mankind is the efficient and economical feeding of the foster mother of the human family—a subject of unusual scientific interest and complication in that it involves the consideration of requirements for maintenance, gestation, and gain or loss of body substance, as well as for the production of milk of widely differing composition, nutritive cost and value.

In view of the facts that dairy cattle are ordinarily fed under carefully controlled conditions, and that large parts of the rations employed in milk production consist of expensive commercial feeding stuffs, it is both practicable and necessary to conduct the business in accord with the principles of science; and as a means to that end the knowledge of the physiology of nutrition, as it applies to this branch of animal production, has been systematized and simplified, and expressed in convenient tabular form, for the guidance of the milk producer.

The realization of the need for such aids to efficient milk production antedated the present understanding of the principles of nutrition by approximately two generations. With the advance of knowledge in this field, therefore, the prevailing feeding standards have changed from time to time; and inasmuch as evolution in this relation is still in process, the recent course of development of these guides to feeding practice is of present concern.

This paper considers the principles of derivation of the feeding standards which have been most used, or have been most influential in the molding of opinion. Inasmuch as the quantitative nutritive requirements of dairy cattle have not yet been definitely and finally established, the standards are compared and discussed without critical judgment of their quantitative correctness.

The earliest recorded suggestions relating to the scientific feeding of dairy cows seem to have come from Haubner (1) in about 1840, but the first definite feeding standard for milch cows was formulated in 1858 by

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Grouven (2), and was expressed in terms of the crude protein, fat, and carbohydrates of feeding stuffs as measured by chemical analysis.

The Digestible Nutrient System

Wolff, in 1864 (3), took the next long step in advance by expressing nutritive requirements of farm animals in terms of digestible crude protein, fat and carbohydrates; and the influence of this standard is still potent in the present day. Wolff's standard was published annually, without fundamental change, in Mentzel and Von Lengerke's Agricultural Calendar, until 1896. This standard was derived from the results of a large number of experiments by different observers at different times. It was intended to meet the requirements of a good, average dairy cow. It did not distinguish, however, between the maintenance requirement and the production requirement, and did not make any allowance for variations in the milk yield. For these reasons Wolff's standard was strongly criticized by Prof. Julius Kühn (4) of the Halle Experiment Station.

Kühn proposed a feeding standard which was somewhat more flexible than Wolff's since it made separate allowances for maintenance and for milk production, and took into consideration the quantity of milk produced. Kühn's standard was intended not to obtain the maximum yield of milk possible, but the maximum economic yield, taking into account the relative prices of the concentrated feeding stuffs and of dairy products. In Kühn's standard the amide compounds were classified together with the digestible carbohydrates instead of with digestible protein.

In 1897 Dr. C. Lehmann of the Berlin Agricultural High School modified Wolff's feeding standard in such ways as to meet Kühn's major criticisms. The result, the Wolff-Lehmann standard, was published annually, until 1906, in Mentzel and von Lengerke's Agricultural Calendar, and was used throughout the educated world. In this standard the kinds and quantities of nutrients specified were varied in accord with the quantity of milk, on terms as satisfactory as provided by the knowledge of the times.

In 1903 important advance was made by Haecker who as a result of many years of study at the Minnesota Station showed that the nutritive requirements of milk production vary not only with the quantity of milk but also with the quality, and especially with the fat content; and published a digestible nutrient standard (5) embracing this idea.

In 1912 Savage (6) published a modification of Haecker's standard, the principal change being an increase of about 20 per cent in protein requirements. Savage expressed his standard in terms of digestible crude protein and total digestible nutrients.

Morrison's (7) is the latest digestible nutrient standard, and is widely used. For details see the table on pages 158 and 159. The maintenance requirements are from Haecker. Two values are given for crude protein requirement for milk production—the lower being Haecker's and the higher Savage's; and two values are also given for total digestible nutrients, the higher being the average of Haecker's and Savage's values and the lower being the same reduced arbitrarily by 10 per cent.

Kellner's Starch Equivalent System

As a result of energy balance studies by Voit and Pettenkofer (8), Rubner (9), Zuntz (10), Meissl and collaborators (11) and Kellner (12), which developed an understanding of the mutual replacement values of nutrients as sources of energy, it became clear that the digestible nutrients of feeding stuffs do not accurately represent their true nutritive values; and to Kellner is due the credit for the first practical application of this modern scientific knowledge, by means of a feeding standard for cattle published in 1905 (13).

With mature oxen as experimental subjects Kellner determined the terms on which food protein, fat, and carbohydrate are converted into body fat; and with the useful residuum of energy from the metabolism of starch as a measure, he expressed the production values of feeding stuffs in terms of starch equivalents.

In the development of these measures Kellner added starch, straw pulp, sugar, wheat gluten and oil to basal maintenance rations, and by relating the body gains of energy, caused by the addition of these materials, to the energy of the digestible portions of the same, he determined the following body-fat equivalents (14):

1 Kgm. of digestible protein	2233	Cals., equivalent to		235	gms.	body	fat
1 Kgm. digestible starch and crude fiber	2356	"	"	248	"	"	"
1 Kgm. digestible cane sugar	1786	"	"	188	"	"	"
1 Kgm. digestible fat	4503–5681	"	"	474–598	"	"	"

Kellner chose the nutritive energy of 1 kgm. of starch as the unit of reference in the expression of production values. On the assumption that starch is 100 per cent digestible, the production value of 1 kgm. of starch was considered equal to 248 grams of fat, or 2356 Cals., which may be considered as the net energy value of starch for body increase. With this value of carbohydrates as a standard, or as unity, digestible protein has a starch value of 0.95,[1] (235 ÷ 248) and digestible fat has a starch value varying

[1] Kellner gave the fraction as 0.94 and so it has been widely quoted. Actually this factor is 0.9476.

from 1.91 to 2.41, depending on the source. The lower value for fat represents the fat of roots and coarse fodders; the higher value represents the fat of oil seeds and oil cakes. The fat of grains and cereal by-products has an intermediate starch value of 2.12.

After having thus established the "production values" of the digestible nutrients, when added in pure, finely-divided form, Kellner proceeded to test the applicability of the factors to ordinary feeding stuffs of cattle. This was done by respiration experiments carried out in exactly the same way as was done with the pure nutrients. A known quantity of the feeding stuff to be tested was added to a basal ration sufficient for maintenance; the digestibility of the added feeding stuff, as well as the quantities of protien and fat produced, were determined by difference; and the total gain of energy was compared with that computed from the digestible nutrients by the use of previously determined factors.

These comparisons showed that with oil meals the "production values" found by direct experimentation agreed closely with the values computed, by the use of the factors, from the quantities of protein, carbohydrate, and fat actually digested. Very different results, however, were obtained with hays and other roughages. With these feeding stuffs the calculated increase in fat did not agree with the observed, the latter being considerably smaller in all cases. Wheat straw showed a difference of 70–80 per cent, oat and barley straws and various varieties of hay showed differences of 30–40 per cent. Kellner accounted for this apparent deficit as resulting from increased work of mastication and digestion, and increased energy loss through fermentation, both being due to the high crude fiber contents of these feeds. He computed that 100 grams of crude fiber diminished the fat production by 14.3 grams, which was equivalent to 58 grams of starch. This correction was considered applicable to hays. For the chaff of various cereals Kellner estimated that the diminution was equivalent to only 29 grams of starch. For green plants, containing 16 per cent or more of crude fiber, the same correction was considered necessary as for dry forage; and if the amount of crude fiber was only 4 per cent or less the correction was the same as for chaff, while between these limits a sliding scale was arbitrarily used. These corrections, based on crude fiber, were applied only in the computation of the production values of roughages from their digestible nutrients.

In the case of concentrated feeds, however, the crude fiber content was found not to be a satisfactory basis for measuring the differences between the observed production values and those computed from the digestible

nutrients by means of the starch equivalents, thus showing that the crude fiber was not the only factor accountable for this difference. In order to account for this discrepancy, therefore, Kellner determined, for a number of concentrates, the observed production value as a per cent of the computed, and considered this percentage as a correction which must be applied to the value computed from the digestible nutrients in order to obtain the true production value. This correction was designated by Kellner as a "Wertigkeit," or evaluating factor. Wheat bran, for example, was found to have a "Wertigkeit" of 77.3 per cent. This means that the computed starch equivalent of wheat bran must be multiplied by 0.773 in order to obtain its true production value.

According to Kellner's system of computing the energy values of feeds it is therefore necessary to compute the starch equivalent from the digestible nutrients and then to correct these values by the employment of special evaluating factors characteristic of the type or class of feeding stuff. In case of roughages a minus correction is made, based on the total crude fiber content of the feed, while in the case of concentrates the percentage correction may be plus or minus. Such percentage corrections were directly determined by Kellner for only a relatively small number (twelve) of concentrates. For the majority of feeding stuffs they were assumed.

Kellner's production values, therefore, are in a sense net energy values, but the method of computing them is highly conventional.

As a basis for the utilization of these starch values in the formulation of a feeding standard for milk production Kellner (15) compared the economy of utilization of feed energy for fattening and for milk production, and found that the latter is decidedly the more efficient function. Kellner's results as recomputed by Armsby (16) show that the utilization of the metabolizable energy for milk production, for three cows, was 68.4 per cent, 72.8 per cent, and 66.9 per cent, respectively, while the corresponding values for fattening were 48.0 per cent, 46.4 per cent, and 43.8 per cent. This finding was corroborated by later investigators, as will be brought out hereinafter.

In these experiments Kellner obtained a good agreement between the computed starch values of the food available for milk production and the starch values computed from the milk constituents, on the basis of the assumption that milk protein and milk sugar are produced from the digestible protein and digestible carbohydrate, respectively, of the food, without any expenditure of energy, and that milk fat is produced from carbo-

hydrate at the same energy expense as is body fat. In accord with this assumption Kellner determined that 1 kgm. of milk fat has a starch value of 3.9 kgm; that 1 kgm. of milk sugar has a starch value of 1.05 kgm; and that 1 kgm. of milk protein has a starch value of 0.94 kgm.

On the basis of these factors, and on the assumption of an average composition of milk of 3.2 per cent fat, 4.6 per cent lactose and 3.3 per cent protein, Kellner computed that for the production of each 10 kgm. of milk 2.0 kgm. of starch values are required. He also estimated that for milk of a high fat content as much as 2.7 kgm. starch value may be required per 10 kgm. of milk.

Kellner estimated the maintenance requirement of the dairy cow on the basis of his results with oxen. For the latter he found that 2 kgm. of starch value represented the average daily maintenance requirement for 500 kgm. of live weight. For cows he considered it necessary to allow 0.5 kgm. additional starch value as a factor of safety, thus making in all 2.5 kgm. of starch value per 500 kgm. live weight.

Kellner expressed the protein requirement in terms of digestible true protein. His estimate for maintenance was 0.3 kgm. per 500 kgm. live weight, and for milk production 0.55–0.65 kgm. of digestible true protein per 10 kgm. of milk.

Kellner utilized these conclusions in the formulation of his latest feeding standard for dairy cows, stating the requirements for maintenance and for milk production together, per 1000 kgm. live weight, as follows:

Milk yield per day per 500 kgm. live weight kgm.	Starch value kgm.	Digestible true protein kgm.
5	7.8–8.3	1.0–1.3
10	9.8–11.2	1.6–1.9
15	11.8–13.9	2.2–2.5
20	13.9–16.6	2.8–3.2

It is apparent that in this standard of Kellner the allowances for milk production are on the basis of his estimates given above, *viz.* 2.0 to 2.7 kgm. starch value and 0.55 to 0.65 kgm. digestible protein per 10 kgm. of milk. When these allowances for milk are deducted from the total allowances, the quantities left for maintenance are 5.8 kgm. starch value and 0.5 to 0.7 kgm. digestible true protein per 1000 kgm. live weight.

The protein quota is in accord with Kellner's estimate as given above, *viz.* 0.3 kgm. per 500 kgm. live weight, but the starch value 5.8 kgm. per 1000 kgm. live weight is appreciably above his estimate of 2.5 kgm. per

500 kgm. live weight, which already includes a factor of safety of 0.5 kgm. The basis for this difference is not revealed in Kellner's writings.

Inasmuch as in Kellner's standard the requirements for maintenance and for milk production are stated together and no separate allowances are made for the different grades of milk, as to fat content, this standard is not readily usable for the computation of rations of individual animals.

Armsby's Net Energy System

Armsby (17), with the same fundamental conceptions as Kellner's, first expressed all of the factors of the process of utilization of food by cattle in terms of calories, and developed the point of view which has become known as the net-energy system. In 1917 he (16) formulated a feeding standard for the milch cow expressed in terms of true protein and net energy.

There is a close similarity between Armsby's net-energy values and Kellner's production values of feeding stuffs. Both were based on complete energy balances, but Armsby's system is simpler and more direct, and less likely to be misunderstood, since he measured energy values in calories, while Kellner measured the same in terms of matter.

According to Armsby the entire expenditure of energy due to the consumption of a feeding stuff is determined from a comparison of the heat production on a basal ration and the heat production on the same ration plus the feed under investigation. This is called the heat increment of the feed. The net energy of the feed is then obtained by subtracting the heat increment from the metabolizable energy, the latter representing the gross energy minus the energy of the feces, urine and methane.

Roughages are found to contain relatively less net energy than do concentrated feeds, not because they contain much crude fiber which causes much mechanical work, and not necessarily because their heat increment values are higher than in the case of concentrates, but because the heat increment value is subtracted from a much smaller amount of metabolizable energy.

Since the direct determination of net-energy values of feeding stuffs is necessarily slow and expensive Armsby and Fries (18) developed, from respiration calorimetric experiments, the following factors for computing metabolizable energy from the digestible organic matter of feeds, these factors to be employed in deriving provisional net-energy values for temporary purposes:

For roughage........................1.588 Therms per pound of digested organic matter.
For grains and similar feeds
With less than 5% digestible fat.......1.769 Therms per pound of digested organic matter.

plying this factor to the maintenance values computed by the "difference procedure" from submaintenance rations is in itself proof that the factor 0.826 is not applicable to the fasting data. In other studies with cows Forbes and associates (24) have compared the efficiency of utilization of food energy for body increase with the utilization of the same for maintenance—the latter involving the use of the directly determined fasting metabolism,—and found an average relationship of 1 for maintenance to 0.761 for body increase. Obviously, it is not possible to have one conversion factor applicable to different planes of nutrition.

Møllgaard also believes that the net energy of the true protein quota for maintenance, and the total net energy quota for the same, should be in the proportion of 1 to 10.

Møllgaard's expression of protein requirements for maintenance and for milk production as net energy of digestible true protein, as has been explained, renders this system somewhat complicated and difficult to use.

On several accounts it is most convenient, and otherwise desirable, that the allowance of protein should be made separately from the quota for energy production. Cows adapt themselves with no apparent disadvantage to wide differences in protein intake; and the allowance of protein may properly be modified in accord with its biological value and in accord with the relative cost of feeding stuffs. It is therefore questionable whether there is an advantage in Møllgaard's method of expression, in which energy-producing nutriment and protein are assigned in combination, and in which the quantity of protein is obscured, for most persons, by the method and terms of its expression; also, as Halnan (31) pointed out, it is technically incorrect to assign energy and protein for maintenance on the same basis, as they are by Møllgaard's standard, since the former is computed in relation to body surface while the latter should be computed in relation to body weight.

Hansson's Food Unit System

The early Scandinavian systems of estimating comparative values of feeds for milk production were based on feeding trials without chemical control; thus Fjord (32), in 1887, initiated a "food unit" system in which the measure was the nutritive value of 1 kgm. of barley; and in Denmark a system came into vogue in which the measure was the nutritive value of 0.5 kgm. of mixed oats and barley (1:1); but in 1915 the Danish, Norwegian and Swedish control unions agreed upon 1 kgm. of barley as the standard unit.

The development of a scientific feeding standard for dairy cows, based on the food unit system, is due to Nils Hansson. As early as 1902 Hansson (33) estimated from results of practical experiments in Sweden that, for each 3 kgm. of average milk produced, 1 food unit in excess of the maintenance requirement is required. At a later date (34) he obtained the same average from control experiments involving over 110,000 cows, the average percentage of fat in the milk being close to 3.5. From the composition of milk containing 3.5 per cent fat Hansson calculated that 1 kgm. of milk contains, as an average, 700 Cals. Hence 1 food unit is equivalent to 3×700, or 2100 Cals. This value is considered by Hansson as representing the net energy value of a Swedish food unit for milk production; and the normal digestible protein content of a food unit for milk production is considered to be 135–150 grams.

From a comparison of Kellner's tables of starch values and his own tables of food units Hansson (34) computed that 1 food unit is equal to 0.7 kgm. of starch value. Since 1 kgm. starch value is equivalent to 2356 Cals. net energy for fattening, 1 food unit is computed to be equivalent to 2356×0.7, or 1650 Cals., of net energy for fattening. The difference between 1650 Cals., for fattening, and 2100 Cals., for milk production, computed as above, is explained by Hansson in the following manner:

According to Kellner 1 kgm. of digestible protein has a fattening value equal to 235 grams of fat, or $235\times9.5=2233$ Cals. net energy,—indicating that the utilization of the energy of the protein is 39.1 per cent ($2.233 \div 5.71$). Hansson found in his experiments with cows that about 75 per cent of the digestible protein is recovered in the milk—which is 36 per cent greater than the utilization of the energy of the same for body increase. Hansson further estimated that in 1 food unit, containing 135 grams digestible true protein, the difference of 36 per cent in the utilization of protein, is equivalent to 278 Cals. ($5.71\times135\times0.36$). Since 278 Cals. do not fully account for the difference between 1650 and 2100 Cals. (that is, the difference between the net energy of a food unit for fattening and for milk protion), Hansson ascribed the remaining difference to a greater utilization of the carbohydrates in milk production.

The above considerations led Hansson to assume that in the process of milk production the percentage utilization of protein is the same as that of carbohydrates, and that the starch equivalent of protein for milk production is therefore determined by the relation of the total energy of 1 gram of digestible protein (5.71 Cals.) to the energy of 1 gram of carbohydrates (4.0 Cals.), that is, it is equal to 5.71/4.0 or 1.43. On this basis

Hansson modified Kellner's computation of starch values by substituting the factor 1.43 for Kellner's factor 0.94 for protein, and leaving the factors for carbohydrates and for fats unaltered. The starch value of a food unit thus computed by the use of the new factor for protein is designated by Hansson as milk production value. The computation of the final milk production value involves also the use of a percentage correction (Wertigkeit) as does the computation of the starch values according to Kellner. In accord with this method Hansson computed the milk production values of Swedish feeding stuffs.

From a comparison of the computed milk production value with the Swedish food units Hansson estimated that, as an average, one food unit corresponds to 0.75 kgm. milk production value. Since 1 food unit is computed to be equivalent to 2100 Cals. net energy for milk production, 1 kgm. milk production value corresponds, according to this computation, to 2800 Cals. net energy for milk production.

Hansson's system of calculating values of feeds for milk production in the above manner was criticized by Fingerling (35) and by Møllgaard (36) mainly on the ground that the energy of protein is assumed to be completely metabolizable, and is defended by Kleiber (37) as being in agreement with results of practice.

The main substance of Hansson's feeding standard for dairy cows is given in the table on pages 158 and 159.

Forbes' and Kriss's Method of Derivation of a Feeding Standard for Dairy Cows

The most recent contribution to this problem has come from the Pennsylvania Institute of Animal Nutrition. In the light of studies initiated by Armsby, and continued after his death,—embracing complete energy balances with milch cows, and extended studies of fundamental principles underlying net-energy determinations —Forbes and Kriss (38) have devised a new method of application of the net-energy conception, to derive the energy requirements of milk production, optionally, in terms of metabolizable energy or total digestible nutrients, in a manner serving to avoid certain of the serious difficulties involved in direct net-energy determinations.

The studies of Forbes and associates (24, 25, 29, 30) on the energy metabolism of cattle have revealed numerous unsolved problems involved in the determination of net-energy values of individual feeding stuffs; and not only the earlier results of Armsby and Fries (23), but their own later

determinations (30), are characterized by such variability as emphasizes the need for further investigation of the practical application of the net energy conception.

Also these studies have removed all possible doubt as to the fact that the efficiency of utilization of feeding stuffs for maintenance differs from the same for body increase, and that the efficiency of utilization for body increase differs from the same for milk production.

Further, these studies have shown that no entirely satisfactory methods have as yet been found for determining net-energy values of individual feeding stuffs for maintenance, for body increase, or for milk production.

In the light of these observations Forbes and Kriss (38) have arrived at the conclusion that the net energy conception applies much more satisfactorily in the evaluation of approximately complete rations than to the determination of the energy values of individual feeding stuffs; have considered the problem of the method of application of this principle to the derivation of feeding standards; and by the use of three energy relationships have devised a new method and principle for utilizing the results of complete energy balances in the formulation of guides in feeding practice.

These three energy relationships are (1) the utilization of metabolizable energy for milk production, (2) the utilization of metabolizable energy for body increase, and (3) the relation between the metabolizable energy of approximately complete rations and the "total digestible nutrients" (the sum of the digestible crude protein, the digestible carbohydrate, and the digestible ether extract multiplied by 2.25) of the same rations.

Regarding the above-mentioned energy relationships the following facts were established:

1. The utilization of the metabolizable energy of normal, mixed rations for the purpose of milk production, as determined in three complete energy balances by Kellner, and in eleven such at the Pennsylvania Institute of Animal Nutrition, was found to agree very well—the average of the 14 determinations being 69.3 per cent.

2. The utilization of the metabolizable energy of seven, normal, mixed rations for the body increase of cattle (steers and cows) was found in 32 determinations at the Pennsylvania Institute of Animal Nutrition, to average 58.4 per cent, with a coefficient of variation of 5.83 ± 0.51 per cent; and in seven such determinations with cows alone, on rations all of the same character, the utilization averaged 57.5 per cent.

3. The relation of the metabolizable energy of normal mixed rations to the total digestible nutrients of the same, was found, in 77 respiration ca-

lorimetric experiments at the Pennsylvania Institute of Animal Nutrition to be remarkably close. The average metabolizable energy per pound of total digestible nutrients was 1.616 Therms, with a coefficient of variation of 3.57 ± 0.27 per cent.

On the basis of the foregoing observations Forbes and Kriss have proposed to determine and to express the energy requirements of the dairy cow in the following manner:

The gross energy of the milk is considered to be the measure of the net-energy requirement of milk production, and is related to the feed required to produce it, in terms of metabolizable energy, as a percentage thereof. In other words, the metabolizable energy requirement for milk production may be obtained by dividing the gross energy of the milk (which may be estimated from the fat content) by the established average percentage utilization of metabolizable energy for milk production of normal mixed rations (69.3 per cent). For convenience in the computation of rations the metabolizable energy requirements for milk production are then computed to equivalent quantities of total digestible nutrients by the use of the experimentally established factor representing this relation in normal mixed rations (1.616 Therms = 1 pound total digestible nutrients).

The maintenance requirement of the animal is determined, in terms of metabolizable energy, as the total heat production of the animal, on a plane of energy equilibrium, on a normal mixed ration. This is accomplished in a single, simple, direct experiment, with a proper accounting for such small gain or loss of energy as occurs. The average of seven such direct determinations, with four cows, was found to be 8.487 Therms per 1000 lbs. live weight. For convenience in the computation of rations the metabolizable energy requirement for maintenance, like the metabolizable energy requirement for milk production, was computed to an equivalent quantity of total digestible nutrients.

The authors of this system state that inasmuch as the maintenance value was determined in a respiration calorimeter it will be revised, presumably by increase, as more data are available, and as a definite basis shall be found for rendering it applicable to conditions of practice.

In their recently formulated feeding standard Forbes and Kriss express the protein requirement in terms of digestible crude protein. The maintenance quota is 0.6 pound per 1000 pounds live weight, while for milk production an allowance is made of 1.25 to 1.75 times the estimated protein of the milk, this latitude being indicated in recognition of various conditions of practice which are enumerated. The protein requirement for milk pro-

duction is, in reality, a phenomenally elastic value. It varies between a minimum of 1.0 times the milk protein, and, according to Perkins,[3] an optimum of probably more than 3.0 and less than 6.0 times the same. In practice the quantity of protein given is much influenced by economic considerations.

The tentative standard of Forbes and Kriss, presented to illustrate this method, is included in the table on pages 158 and 159.

A fundamental difference between this system of Forbes and Kriss and those of Armsby and Møllgaard is that while in the latter the net-energy requirements for milk production are converted by the use of experimentally determined factors to net-energy equivalents for fattening, in the system of Forbes and Kriss the net-energy requirements for milk production are converted by the use of experimentally determined factors to the equivalents of the same in terms of total digestible nutrients.

The net-energy and the starch-equivalent values of feeding stuffs are theoretically much more significant than are digestible nutrients, but, on the basis of present knowledge, digestible nutrients can be much more accurately determined than can net-energy and starch-equivalent values; and in the use of the digestible nutrient values, by the method of Forbes and Kriss, the digestible nutrients are related to the milk energy by a computation which adequately recognizes the energy losses between digestible nutrients and metabolizable energy, and between metabolizable energy and net energy. The system of Forbes and Kriss has the further advantages of being the simplest system; of employing more satisfactory measures of protein values and of energy requirements for maintenance; and of expressing nutritive requirements in measures with which all students of animal feeding are acquainted.

Conversion Factors for Measures of Nutritive Value

The quantitative relations between the feeding standards which have been discussed may be determined by means of the following conversion factors:

1 milk unit is equivalent to 1 Therm milk energy or 0.837 Therm net energy for fattening. (Møllgaard)

1 Kgm. digestible true protein is equivalent to 2.233 Therms net energy for fattening. (Kellner, Møllgaard)

1 Kgm. starch value is equivalent to 2.356 Therms net energy for fattening. (Kellner)

1 food unit is equivalent to 1.650 Therms net energy for fattening. (Hansson)

1 food unit is equivalent to 2.100 Therms milk energy. (Hansson)

[3] Personal letter to E. B. Forbes, of the date of January 23, 1931.

MAINTENANCE REQUIREMENTS, PER 1000 POUNDS LIVE WEIGHT PER DAY

Author	Energy			Protein	
	In original terms	Net energy (for fattening)	Total digestible nutrients	Digestible true protein	Digestible crude protein
		Therms	Pounds	Pounds	Pounds
Kellner (1912)	2.631 kgm. starch value*	6.200	6.673	0.5–0.7*	
Armsby (1917)	6.0 Therms net energy	6.000	6.456	0.5	0.6**
Møllgaard (1929)	5.446 Therms net energy	5.446	5.860	0.54	
Nils Hansson (1926)	3.175 food units	5.239	5.638	0.5	
Morrison (1923)	7.925 pounds total digestible nutrients	7.362	7.925		0.7
Forbes and Kriss (1931)	5.260 pounds total digestible nutrients	4.888	5.260		0.6

REQUIREMENTS FOR MILK PRODUCTION, PER POUND OF MILK

Author	Per cent fat in milk	Energy			Protein	
		In original terms	Net energy (for fattening)	Total digestible nutrients	Digestible true protein	Digestible crude protein
			Therms	Pounds	Pounds	Pounds
Kellner (1912)	all grades	0.091–0.122 kgm. starch value*	0.214–0.287	0.230–0.310	0.055–0.65*	
Armsby (1917)	3.0	0.214 Therms net energy	0.214	0.230	0.043	
	4.0	0.265 " " "	0.265	0.285	0.049	
	5.0	0.315 " " "	0.315	0.339	0.055	
	6.0	0.361 " " "	0.361	0.388	0.061	
	7.0	0.408 " " "	0.408	0.439	0.068	

REQUIREMENTS FOR MILK PRODUCTION, PER POUND OF MILK (*Cont.*)

Author	Per cent fat in milk	Energy: In original terms	Energy: Net energy (for fattening)	Energy: Total digestible nutrients	Protein: Digestible true protein	Protein: Digestible crude protein
			Therms	Pounds	Pounds	Pounds
Møllgaard (1929)	3.0	0.281 milk units	0.235	0.252	0.046	
	4.0	0.336 " "	0.281	0.302	0.056	
	5.0	0.390 " "	0.326	0.351	0.064	
	6.0	0.440 " "	0.368	0.396	0.073	
	7.0	0.485 " "	0.406	0.437	0.080	
Nils Hansson (1926)	3.0	0.15 food units	0.248	0.267	0.040	
	4.0	0.17 " "	0.281	0.302	0.045	
	5.0	0.19 " "	0.314	0.339	0.050	
Morrison (1923)	3.0	0.257–0.286 pounds T.D.N.	0.239–0.266	0.257–0.286		0.047–0.057
	4.0	0.311–0.346 " " " "	0.289–0.321	0.311–0.346		0.054–0.065
	5.0	0.362–0.402 " " " "	0.336–0.373	0.362–0.402		0.060–0.073
	6.0	0.409–0.454 " " " "	0.380–0.422	0.409–0.454		0.067–0.081
	7.0	0.454–0.505 " " " "	0.422–0.469	0.454–0.505		0.074–0.089
Forbes and Kriss (1931)	3.0	0.251 pounds T.D.N.	0.233	0.251		0.038–0.054
	4.0	0.300 " " " "	0.279	0.300		0.045–0.063
	5.0	0.350 " " " "	0.325	0.350		0.050–0.070
	6.0	0.393 " " " "	0.365	0.393		0.056–0.078
	7.0	0.433 " " " "	0.403	0.433		0.061–0.085

* Computed by the writer from the data on page 000, in direct proportion to the live weight, for the purpose of comparison, since in Kellner's standard the requirements for maintenance and for milk production are not given separately.

** Considered by Armsby to be the equivalent of 0.5 pound true protein.

1 Kgm. milk production value is equivalent to 2.800 Therms milk energy. (Hansson)
1 food unit is equivalent to 0.7 Kgm. starch value. (Hansson)
1 food unit is equivalent to 0.75 Kgm. milk production value. (Hansson)
1 Therm metabolizable energy is equivalent to 0.575 Therm net energy for fattening. (Forbes and Kriss)
1 Therm metabolizable energy is equivalent to 0.693 Therm milk energy. (Forbes and Kriss)
1 pound total digestible nutrients is equivalent to 1.616 Therms metabolizable energy. (Forbes and Kriss)
1 pound total digestible nutrients is equivalent to 0.929 Therms net energy for fattening. (Forbes and Kriss)

By the use of these factors such a comparison as suggested above is made in the preceding table, all statements of energy requirements being computed to equivalent quantities of total digestible nutrients, to provide a basis for comparison.[4]

MAX KRISS

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19-4-3

COEFFICIENTS OF DIGESTIBILITY OF THE CONSTITUENTS OF MILK AND THE BALANCE OF CALCIUM AND PHOSPHORUS IN CALVES ON A MILK DIET*

By

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(*From the Kansas Agricultural Experiment Station, Manhattan*)

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IN CONNECTION with work on the influence of the feed received on the nutritive value of the milk produced, two calves were fed on a diet of milk alone. After these calves had been on this exclusive milk diet for a period of several months, a metabolism trial was run in which the coefficient of digestibility of the constituents of the milk and a mineral balance were determined. It is the purpose of this article to give the results of this digestion trial. The question of the deficiences of milk as an exclusive diet for calves will be discussed in another paper.

Handling Previous to the Digestion Trial

Two Holstein calves, No. 17 a male and No. 18 a female, were placed on a diet of milk alone at birth and were continued on this diet for a year or more. The amount of milk given varied from eight pounds of whole mixed herd milk daily at birth to 10 pounds of pasteurized skim milk and 15 pounds of whole mixed herd milk during the trial. For approximately the first six months whole milk alone was used, as much as 20 pounds per calf being fed. The milk was not fed *ad libitum* or according to standards but was limited to the consumption of milk by two calves on another phase of the experiment for which calves No. 17 and No. 18 were serving as controls. At times the amount of the digestible nutrients received by the calves was considerably below the minimum requirements for growing dairy cattle as given by Morrison (1) but in the main the true protein and net energy approximated the requirements as given by Armsby (2). At approximately 8 and 7½ months of age respectively, the digestion trial here discussed was conducted. During the period of the digestion trial the two

* Contribution No. 70, Department of Dairy Husbandry, and No. 156, Department of Chemistry.

calves were receiving 62 and 70 per cent respectively of the minimum requirements for total digestible nutrients as given by the Morrison standard. The calves were stabled in straw bedded stalls and were turned in a dry lot whenever the weather was suitable. They were given free access to water from the college mains while in the lot and were watered twice daily while in the barn. The calves were given access to salt frequently.

Except at feeding time the calves were muzzled with a muzzle shown in Figure 1, made from ¼ inch mesh hail screen with a sheet metal bottom and fitted to a leather halter. When a tendency was noted for the calves to work bedding material through the screen, a lining of ordinary screen wire and later of copper screen wire was inserted in the muzzles.

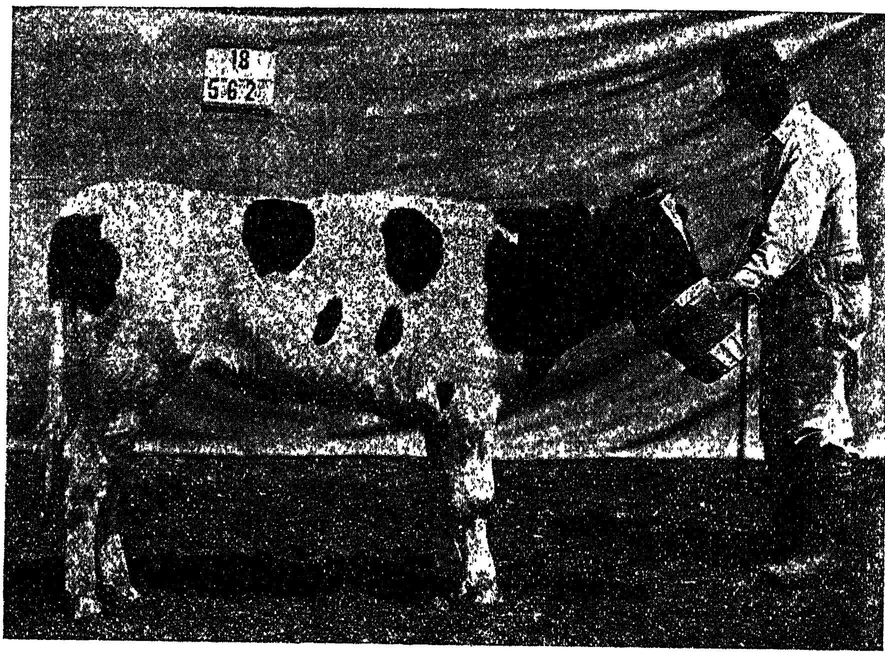

FIG. 1. Showing type of muzzle used.

On Monday and Tuesday of each week the calves were weighed and on Monday they were measured to determine growth, the following measurements being taken: height at withers, chest girth, barrel girth, width of hips, and width of pin bones. Figures 2 and 3 show that the calves at the time of the digestion trial were considerably below normal[1] in body weight.

[1] The normal used is the average weight and height of similar calves of comparative ages fed normally at the Kansas Agricultural Experiment Station.

At the same time they were approximately normal in height of withers as is shown in Figures 4 and 5. This would indicate normal growth of body framework but a lack of body fat and fleshing. Post mortem examination

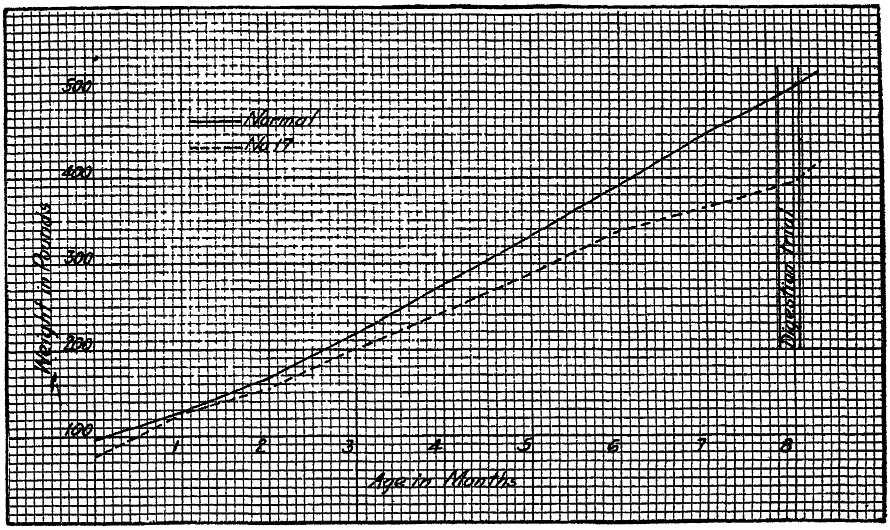

FIG. 2. Showing weight as compared with the normal for Holstein males.

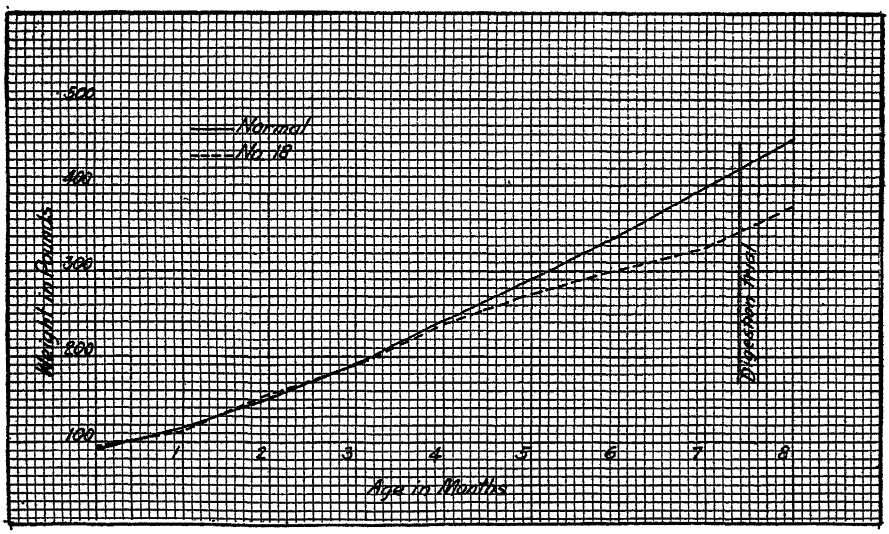

FIG. 3. Showing weight as compared with the normal for Holstein females.

of No. 17 and of other calves on an exclusive milk diet has shown a decided lack in size and tone of the organs of digestion.

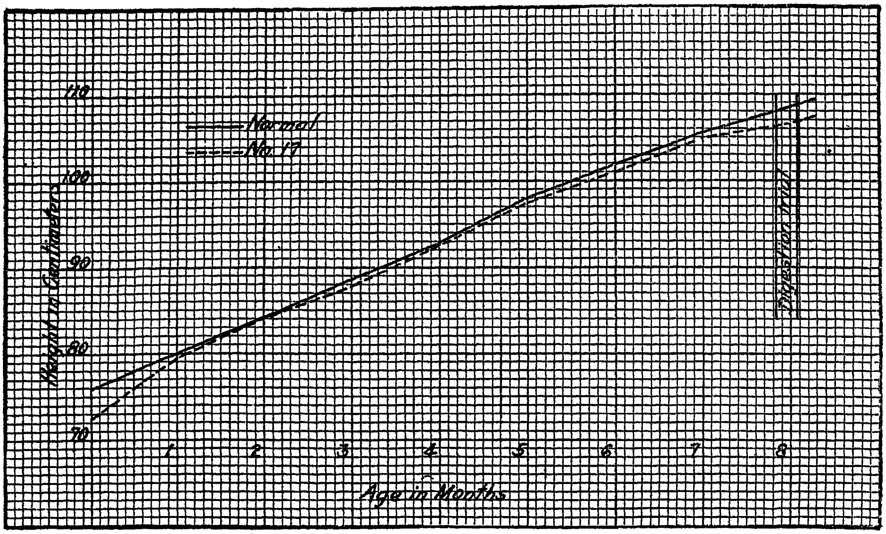

FIG. 4. Showing height of withers as compared with the normal for Holstein males.

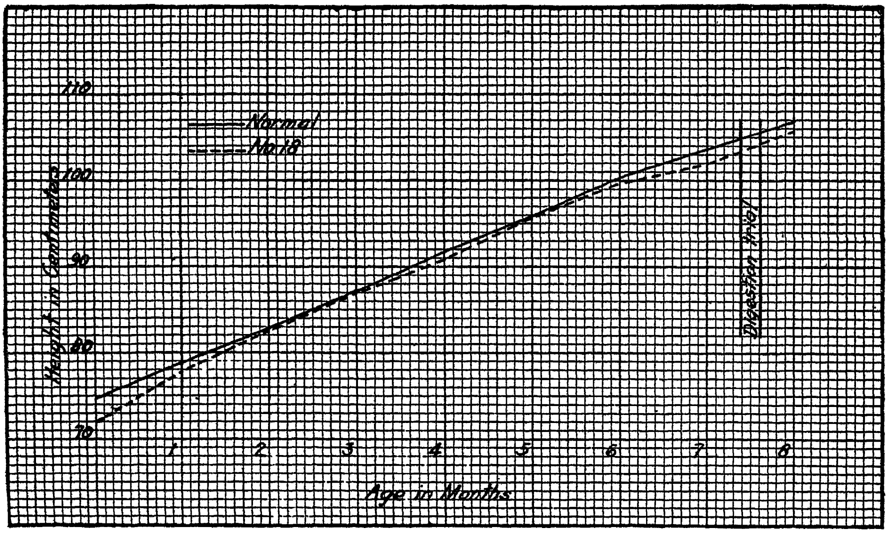

FIG. 5. Showing height of withers as compared with the normal for Holstein females.

Experimental Procedure

The calves were removed from the dairy barn to special stalls in the nutrition laboratory three days before the metabolism trial was begun, so they would become accustomed to the new surroundings prior to the collection of samples. As they were to continue on the same kind of milk they had been receiving, it was not necessary to run a long preliminary feeding period.

The milk used was a mixture of whole milk and centrifuged milk in the ratio of two-fifths of the former and three-fifths of the latter. Each calf was given twenty-five pounds a day. This was divided into two feeds, one-half being given in the morning and the other in the evening. At each feeding time a sufficient amount of whole and skimmed milk was mixed together to feed the two calves and to allow some for sampling. A 100 cc. sample was taken at each feeding and these were composited for the seven-day sample. After each feeding the calves were given all the hydrant water they would drink. The amount of water consumed by each calf was recorded so that the amount of calcium received in the water could be determined.

The collection of the urine and feces was cared for by two attendants who worked in six-hour shifts. The urine of the male calf (No. 17) was collected in the usual manner by means of a rubber funnel, held in place by a suitable harness, and a rubber tube running through the floor to a bottle. The urine of the female calf (No. 18) was caught in a suitable dipper and placed in a bottle. In neither case was there any appreciable loss of material. The twenty-four hour sample from each calf was weighed, thoroughly mixed and an aliquot of one-twentieth taken for analysis. These aliquots were composited for the seven days, chloroform being used as a preservative. The feces were caught in dippers and transferred to wide-mouth bottles containing alcohol. The amount of feces, which was voided on the average of once during the twenty-four hours, was very slight. They were light in color, fairly solid in consistency, and had very little odor. At the end of the seven-day period the alcohol was evaporated off and the residue was brought to dryness in an oven heated to 100 degrees C. The entire sample for each calf, 374.2 grams for No. 17 and 396.6 grams for No. 18 was then ground and sampled for analysis. The amounts of milk and water consumed and of urine and feces voided are shown in Table I.

Calf No. 18 consumed very little water as compared with No. 17 due perhaps to the change of environment during the metabolism trial.

The analyses of the milk, water, urine, and feces were carried out by the

TABLE I

GRAMS OF MILK AND WATER CONSUMED, AND URINE AND FECES VOIDED DURING SEVEN-DAY METABOLISM TRIAL

Calf No.	Grams of milk consumed	Grams of water consumed	Grams of feces voided (dry weight)	Grams of urine voided
17............	79,380	44,524	374.2	93.389
18............	79,380	3,101	396.6	54.632

usual standard methods. The results of the analyses are given in the following table:

TABLE II

PERCENTAGE COMPOSITION OF MILK, WATER, URINE AND DRIED FECES

	Milk	Water	Urine No. 17	Urine No. 18	Feces No. 17	Feces No. 18
Nitrogen............	0.56	—	0.268	0.481	5.07	5.82
Ether ext...........	2.26	—	0.0	0.0	7.11	4.31
Sugar...............	4.20	—	0.0	0.0	nil	nil
Crude fiber..........	—	—	—	—	1.69	0.81
Ash.................	0.709	—	0.3165	0.5886	25.88	28.04
Calcium.............	0.108	0.0151	nil	nil	9.17	8.02
Phosphorus..........	0.0908	nil	0.0329	0.612	0.472	0.464

A small amount of crude fibre appeared in the feces due in part, no doubt, to the fact that the calves were working constantly to get bits of straw or shavings into their mouths and occasionally they were successful in doing so.

Table III shows the balance of nutrients during the seven-day metabolism trial.

The relatively low retention of nitrogen, 39.4 per cent for No. 17 and 35.7 per cent for No. 18, is no doubt due to the use of protein for energy. The twenty-five pounds of milk, which each calf received per day, made up of a mixture of two-fifths whole milk and three-fifths centrifuged milk, furnished slightly more protein than was required for normal growth, but did not furnish sufficient net energy, so the energy rather than the protein was the limiting factor in determining growth.

The results show that the two calves retained on the average 8.0 grams of calcium and 5.3 grams of phosphorus per day.

TABLE III

BALANCE OF NUTRIENTS DURING THE SEVEN-DAY METABOLISM TRIAL

	Calf No. 17				Calf No. 18			
	Grams fed	Grams eliminated	Balance	Per cent retained	Grams fed	Grams eliminated	Balance	Per cent retained
Nitrogen....	444.5	269.3	+ 175.2	39.4	444.5	285.9	+ 158.6	35.7
Ether extract	1794.0	26.6	+1767.4	98.3	1794.0	17.1	+1776.9	99.0
Ash.........	562.8	392.4	+ 170.4	30.3	562.8	432.8	+ 130.0	23.1
Calcium.....	92.4	34.4	+ 58.1	62.8	86.2	31.8	+ 54.4	63.1
Phosphorus..	72.1	32.5	+ 39.6	54.9	72.1	35.2	+ 36.9	51.2
Sugar.......	3334.0	0.0	+3334.0	100.00	3334.0	0.0	+3334.0	100.0

TABLE IV

COEFFICIENT OF DIGESTIBILITY OF MILK WHEN USED AS AN EXCLUSIVE DIET FOR CALVES

Nutrient	No. 17			No. 18		
	Grams in feed	Grams in feces	Coefficient of digestibility per cent	Grams in feed	Grams in feces	Coefficient of digestibility per cent
Nitrogen..........	444.5	19.0	95.7	444.5	23.1	94.8
Ether extract......	1794.0	26.6	98.5	1794.0	17.1	99.0
Sugar.............	3334.0	nil	100.0	3334.0	nil	100.0

Henry and Morrison (3) give the following as the average coefficient of digestibility of milk by calves: Crude protein, 94 per cent; nitrogen-free extract, 98 per cent; fat, 97 per cent. A comparison of these average results with the results in Table IV shows clearly that the ability of the calves to digest food was not impaired by restricting them to a milk diet for a period of about eight months. This is of interest in view of the fact that it is generally believed that roughage is necessary in the diet of calves. No doubt roughage plays a part in the development of the muscles of the paunch and intestines. These results, however, indicate that this development is not necessary for the secretion of the digestive juices.

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THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

I. STUDIES WITH THE VITAMIN A OF BUTTER FAT

By

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IN VIEW of the fact that many of the natural edible fats are soluble in medicinal mineral oils and conversely that the mineral oils are soluble in the fats, one may well be puzzled as to the probable alimentary fate of a third substance mutually soluble in the first two when all three are administered together orally to an animal. It is rather generally accepted that hydrocarbons such as mineral oils, contrary to the behavior of the natural dietary fat, are not absorbed unless perhaps in very small and ordinarily rather inappreciable amounts (cf. Bradley and Gasser, 1911; Bloor, 1913; Mellanby, 1927, and Channon and Collinson, 1929). The question, then, arises: How is this third substance, *e.g.*, one of the vitamins contained in fat, ultimately distributed in the alimentary tract between mineral oil, which finally passes unabsorbed out of the gut, and the fat which for the most part undergoes digestion and eventual absorption through the intestinal wall into the body? It seems logical that mineral oil might dissolve and retain significant though minute quantities of important accessory substances such as the fat-soluble vitamins. Should this occur, the body might be deprived of a portion of its indispensable materials, perhaps even suffering encroachment upon the absolute minimum necessary for health and well-being. Similarly, although surely much more improbably, mineral oil might cause such depletion by chemically inactivating the necessary substance, *e.g.*, vitamin A. These conceptions lead to the recognition of a possible limitation in the very large consumption of mineral oil as a therapeutic agent in the treatment of constipation, a limitation quite aside from the mineral oil characteristics of being apparently pharmacologically innocuous and non-habit forming. As has been pointed out in the editorial columns of the Journal of the American Medical Association (1927), the problem, on account of its general importance, deserves a thorough investigation.

A few brief reports concerning the effect of mineral oil administration to animals under observation with respect to their conservation of vitamins A and D have already been published. Burrows and Farr (1927) found death to occur with little delay among rats ingesting diets containing butter fat at a 17 per cent level, and mineral oil in the rather excessive proportions of 8 to 25 per cent. Study of their data leads one to the conclusion that the experimental animals died from factors associated, indeed, with the very large intake of mineral oil but *not* through the secondary production of a vitamin A deficiency. Dutcher, Ely, and Honeywell (1927) observed that mineral oil mixed with butter fat may prevent a considerable portion of the vitamin A in this butter fat from being available to rats previously exhausted of their vitamin A reserve. Failure of growth resulted whether daily doses of 67, 133, or 200 mgm. of butter fat—diluted to a total of three and one-half times the original weight with mineral oil—were given. Xerophthalmia developed in those animals receiving the smallest allotments of the butter fat-mineral oil combination. However, 67 mgm. of butter fat alone were sufficient to permit growth and to prevent the onset of eye lesions. More recently Ely, Honeywell, and Dutcher (1929) reported confirmation of their former findings. Again, Moore (1929) investigating carotene as a source of vitamin A noted that: "Poor growth responses . . . were given in some cases, and notably in those in which the carotene was administered in medicinal paraffin." And Hawk, Levine, Stucky, and Oser (1929) state: "Data from curative experiments on rats depleted of their bodily reserves of vitamin A, indicate that mineral oil lowers the utilization of this vitamin in cod liver oil." These citations would appear, perhaps, to constitute a considerable indictment of mineral oil therapy. Yet it must be borne in mind that to mix mineral oil at once and intimately with the entire daily vitamin A ingestion is contrary to ordinary human practice.

Moness and Christiansen (1929), on the other hand, decided that liquid petrolatum (Squibb) is "unobjectionable" and "even suitable as a vehicle for vitamin administration." Furthermore, Marcus (1929), possibly uncritical with respect to our present theme, proposed the use of mineral oil as a protective solvent for vitamin A; and Collison, Hume, Smedley-Maclean, and Smith (1929) with some success essayed its use as a carrier likewise for vitamin A, although the latter authors remark that "the effect seemed rather better with the hardened cotton-seed oil." They refer also to a finding of Hume and Smith (unpublished) that a certain fraction of an extract of spinach "is an adequate source of vitamin A, when dis-

solved in liquid paraffin." Finally, the experiments of MacDonald, Andes, and Briggs (1930), with mineral oil as an extraction agent in connection with milk, have a bearing on the point in question. On the basis of colormetric tests, they conclude: "The results show no decrease in vitamin A content because of mineral oil treatment for the removal of the onion flavor and odor from milk."

In our own investigation dealing with the general question of the effect of mineral oil on the nutritional economy of the fat-soluble vitamins, we elected first to repeat the experiments of Dutcher *et al.*, in which the butter fat was fed at the 67 mgm. daily dosage. In Chart 1 are presented protocols of our results which substantiate the findings of these authors in that 167 mgm. of mineral oil (in our case, two well-known medicinal brands) when mixed with an adequate but marginal 67 mgm. intake of butter fat, considerably decrease the availability of the vitamin A.

We have been interested chiefly, however, in determining the influence of mineral oil administered to experimental animals under conditions approximately those customary in the human usage of this therapeutic agent. Particularly have we aimed to study the effect exerted by mineral oil when introduced into the alimentary tract not incorporated with the butter fat but at certain time intervals reasonably distant from the dispensing of the vitamin A-bearing substance.

Plan of Experiments

Animals. Since most of the available information concerning vitamin A has been obtained through use of the rat, the present study was carried out with this species. Not only is vitamin A required by both rat and man, but its deficiency leads to many similar symptoms in the two forms. Furthermore, the action of mineral oil obviously must be localized in the alimentary tract and here again we find no marked difference in anatomy and functioning. Both the rat and man are omnivorous. Thus the rat would appear to provide fairly comparable alimentary conditions for the mixing and intermingling of the food mass, the vitamin in question and the mineral oil. Both male and female rats were used in the first experiments; subsequently only males were employed.

Diet. A conventional diet described in Table I was formulated for use in depleting the fats of

Table I

Composition of the Basal Diet (Without Added Vitamin A)

	Total Weight	Total Calories
	per cent	per cent
Casein, extracted	18	16
Starch, cooked	63	55
Salts, Osborne and Mendel (1919)	4	—
Hydrogenated vegetable oil*	15	29
	100	100

* Crisco.

Vitamins B, G, and D were supplied daily apart from the rest of the ration in the form of 400 mgm. of dried yeast (Northwestern Yeast Co.) irradiated according to Hess (1927).

vitamin A and subsequently in the testing of the butter fat given with and without mineral oil. Large quantities (25 kg.) of casein were prepared at one time. Each batch (1000 gm.) of technical casein was extracted successively with 3000 cc., 2500 cc., and 2500 cc. of boiling 95 per cent alcohol (cf. Osborne and Mendel, 1921) for one, one and one-half, and two hours respectively. The mother liquor of the third extraction of one batch was used for the first extraction of the next. Each extraction was immediately followed by filtration with suction and by a washing with 500 cc. of alcohol. The material was then extracted for one hour in 2500 cc. of boiling ether, filtered, then dried in air and finally heated for 48 hours in an oven at 100° C. The starch was prepared by pouring a cold suspension of cornstarch[1] into boiling water with vigorous stirring for a few minutes in order to cause complete gelatination. The resulting product was then dried and ground. Fat, an important constituent of human food, was included in the diet for two reasons: first, because it has been claimed by various writers that some fats under certain conditions exert a destructive action upon one or another of the fat-soluble vitamins, and secondly, because it appeared probable that fat in the intestine would ordinarily compete with the mineral oil for the vitamin A. In order to have such destructive action and such competition operating in a normal manner, whatever that might be, we endeavored to duplicate the fat content of a liberal American dietary[2]—about 30 per cent of the total calories according to Murlin and Hildebrandt (1919). Rats ingesting this basal diet developed eye symptoms in the minimum time of 18 days and in the average time of 24 days, with growth ceasing in the majority of animals shortly after the eyes became involved.

The preparation, dosage, and administration of the mineral oil and butter fat. Several pounds of creamery butter[3] were melted and allowed to separate in the usual manner whereupon the fat was filtered through a hot-water funnel. The material, placed in 300 cc. bottles, was stored in a container charged with carbon dioxide gas and kept in the refrigerator. About once a month a convenient amount was removed and divided into portions sufficient for a week. At the beginning of each week the old supply was discarded for a new one, so that no daily sampling was done after the butter fat had been melted more than seven or eight times. All lots of material in current use were kept in the dark and at about 5° C. In dispensing the butter fat, the practice was to warm it to a temperature of 50° C. and then to draw it into a special pipette designed and calibrated to deliver drops of 25 ± 0.5 mgm. Delivery was made onto inverted, clean and dry crucible lids from which the ring handles had been removed. These fat portions were then allowed to chill in the ice box until time for distribution among the experimental animals. Assay, involving the effect on growth and eye condition of the rat, revealed that the butter fat preparation just described was inadequate when supplied in 50 mgm. daily dosage, more nearly adequate at a 75 mgm. level and approximately adequate at a 100 mgm. level.

All the mineral oil-vitamin A experiments so far reported in any detail

[1] Duryeas' brand.

[2] The data for computation of this average were secured at various United States Army Training Camps during the World War.

[3] "Sunlight" brand.

have dealt with the rather indefinite substance "mineral oil." We employed two commonly used standard brands of material, representative of the paraffin and naphthene types of petroleum. These, we shall henceforth call Mineral Oils No. 1 and No. 2.

Choice of the amount of oil to be given to a rat was governed by two considerations: 1.—comparison with the human therapeutic dose and 2.—the quantity necessary to produce a detectable effect on both the character of the feces and the motility of the gut. Inasmuch as mineral oil presumably escapes absorption and, therefore, is confined in its action to the alimentary tract, we could find no compelling reason for comparing human and rat dosages, as some have done, on the basis of body weight. It appeared far more reasonable to make the comparison on the basis of the gut capacity or food content calculated from the caloric intake. The approximate rat:man weight ratio is 250:75,000. The approximate caloric ratio is 50:3000. Fantus (1920) gives the adult human dose as 15 to 90 cc. If we choose 30 cc. (two tablespoonfuls) as a reasonable amount to be taken continually (daily) over a long period of time, the weight comparison indicates 30/300 or 0.1 cc. and the caloric comparison, 30/60 or 0.5 cc. as the therapeutic dose for a rat.

It was found that, although a daily intake of 0.2 cc. of mineral oil did not noticeably alter the external appearance of the rat feces, 0.5 cc. of either mineral oil produced a pronounced alteration in their character. They were softer and larger, and often were coated with a thin film of oil, and analysis revealed a greater water content. Furthermore, the 0.5 cc. intake of mineral oil was shown to cause a rather moderate but nevertheless definite acceleration of intestinal motility. Data to support this last statement will be presented in a separate communication.

On the grounds of the foregoing evidence, 0.5 cc. was adopted as the standard rat dose of mineral oil in these experiments. This was given every day except Sunday in three separate 0.17 cc. doses within the following hours: 8 to 9 a.m., 2 to 3 p.m. and 5 to 6 p.m. By means of a 1 cc. insulin syringe, the oil was delivered through a small glass tube well to the rear of the pharynx. The soiled tube was replaced always with a clean one before proceeding with the next animal. It was found that a rat could handle as much as the 0.17 cc. administered in this way without any overflow about the mouth that might result in subsequent loss. The procedure was justified by repeated observation of the animals' habits. Aside from occasionally displaying certain refractory reactions to the mineral oil, the rats took the material well and even, in some instances, soon became accus-

tomed to coöperate in its reception. Again, conclusive evidence that the rat actually ingested the oil given was secured by demonstrating that it could be practically quantitatively recovered from the feces as ether-soluble material.

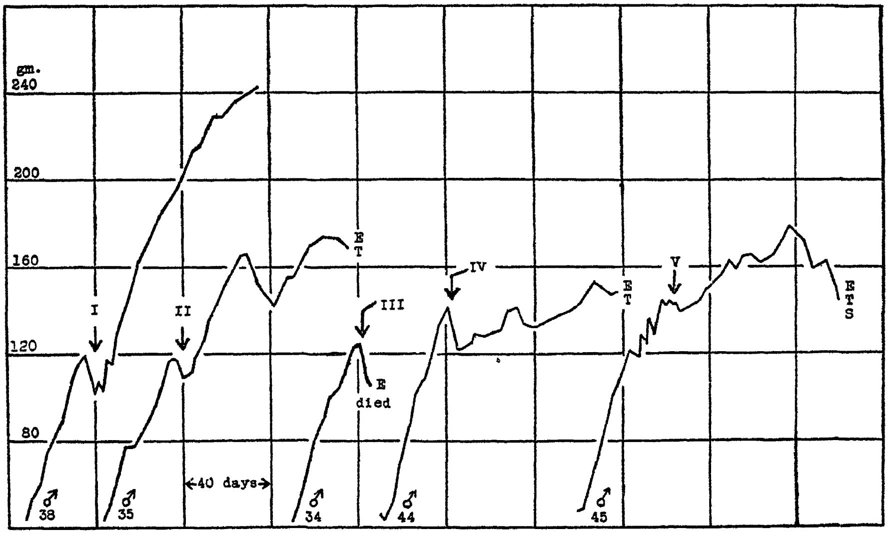
The butter fat was dispensed every day of the week during the hour of 11 to 12 a.m. and was almost invariably immediately and completely consumed. Thus, a stream of mineral oil was kept flowing through the rat's alimentary tract with obvious opportunity for the oil to make contact with other material ingested between the doses of the oil. Yet three hours always elapsed between the butter fat and mineral oil administrations. This program represented what appeared to be a rigorous but fair trial of the action of mineral oil on vitamin A in the gut under conditions similar to those attending the use of mineral oil by man.

Conduct of experiments; indices of vitamin A deficiency. Young albino rats at about the weaning age of 21 days and always within the weight range of 40 to 50 gms. were removed from the mothers and from the stock diet.[4] They were placed in individual cages equipped with large-meshed wire false bottoms, given the basal vitamin-deficient diet, and allowed to grow until definite avitaminosis was evidenced in either eye lesions or lack of growth or both. In case of occasional considerable delay in the development of a positive sign in one of the two indices, the other was accepted as an indication to proceed with the experiment. This plan avoided carrying the deficiency to the point of quick general debacle. Like others, we have also recognized the possibility of depleting animals to the degree that there occurs irreparable damage whose manifestation may be subtle and long delayed. These dangers we have tried to avert by using a standard depletion interval arranged to be slightly less than the minimum time required for unmistakable eye symptoms to occur. We have attempted also to use the depletion time of the animal first becoming deficient as a signal to begin the experiments with the remaining members of the litter. However, it seemed necessary to abandon these procedures in favor of the scheme of definitely depleting each rat. The vaginal smear method, though claimed to provide a sensitive index for the measurement of vitamin A exhaustion in the female rats, was of very little use because in many instances the vagina did not open until several days after other deficiency symptoms had been well established (*cf.* Coward, 1929). Nevertheless, there appeared to

[4] The rats whose growth curves are depicted in Charts 1, 2, and 3 were reared on Sherman's Stock Diet B somewhat modified; the remainder of the animals (See Charts IV and V) on a calf meal diet (Maynard, 1930). Both of these diets were supplemented at particular times by separate administration of cod liver oil, fresh lettuce, dried yeast, etc.

us several possible reasons why the experimental animals to be used in the comparisons that have been outlined here should be in even a more certain state of vitamin balance than that required of animals depleted simply for the purpose of demonstrating the curative power of some natural vitamin A-containing substance. To offset the rapid development of irreversible changes, therefore, each rat after being satisfactorily depleted was given

CHART 1.



EXPLANATION OF CHART 1

The Roman numerals indicate the introduction of butter fat and mineral oil in daily doses as follows:

I. 67 mgm. of butter fat.
II. 33 mgm. of butter fat.
III. No butter fat.
IV. 67 mgm. of butter fat mixed with 167 mgm. of Mineral Oil No. 1.
V. 67 mgm. of butter fat mixed with 167 mgm. of Mineral Oil No. 2.

Letters immediately adjacent to the end of a curve are used to record necropsy findings:

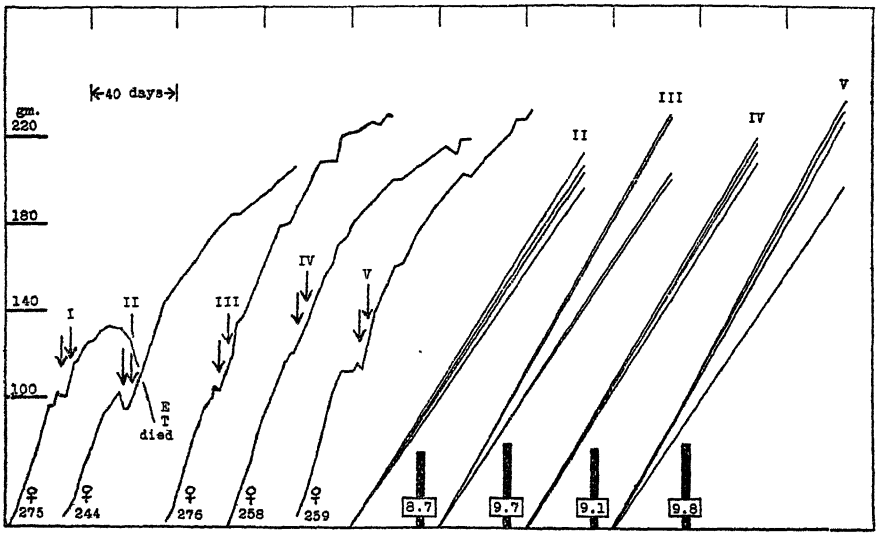
E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.

The growth curves presented in this chart are representative of the results secured in carrying out a repetition of the experiments of Dutcher, Ely, and Honeywell (1927). The basal diet used by these authors both for depleting the rats of vitamin A and for the subsequent test periods contained casein, dextrin and salts but no fat. Every described detail of their experimental plan was accurately duplicated, with the exception that in our experiments, each individual animal was kept upon one régime for the longer term of 75 days. The butter fat which we employed was from a Lot No. 1 which was a different lot than that referred to later in this paper. Fresh mixtures of the butter fat and mineral oils were prepared every twelve days and were always kept chilled and in the dark.

the full experimental dose of vitamin A for four days before it was transferred to one of the experimental régimes.

The animals were divided among the various experimental groups with attention to an equitable distribution as to sex, weight, age, eye condition,

CHART 2.



EXPLANATION OF CHARTS 2 AND 3

The Roman numerals indicate the introduction of butter fat and mineral oil in daily dosages as follows:

I. No butter fat.
II. 75 mgm. of butter fat.
III. 150 mgm. of butter fat.
IV. 150 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 1 *separately* (see text).
V. 150 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 2 *separately*.

Letters adjacent to the ends of the protocol curves represent necropsy findings:

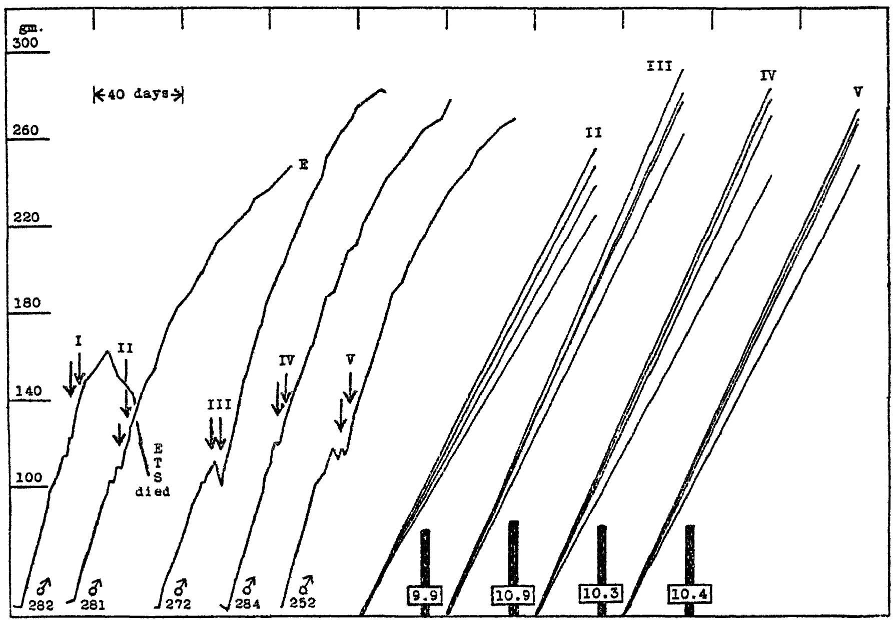
E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.

GENERAL EXPLANATION OF CHARTS 2, 3, 4, AND 5

The growth curves depicted in these charts are of two kinds: 1. representative curves plotted in detail without smoothing to serve as protocols and 2. straight line graphs to facilitate group comparison. The latter were prepared by drawing lines from an arbitrary common origin representing 40 gm. to points representing the final weights (in gm.) attained. The corresponding abscissae represent the average depletion time, 26 or 28 days, plus the recuperation interval, 4 days (see text), plus the experimental period, 75 days; that is, 105 days in Chart 5 and 107 days in Charts 2 and 3. The black columns show averages of group food consumptions in grams. Of the two arrows shown in the protocol curves, the first records the time when the animal was adjudged to be definitely depleted of vitamin A: the second, the end of the four-day readjustment period. The butter fat used in all experiments described in these Charts is from a Lot No. 2, a different lot than that referred to in Chart 1.

etc. The groups may be labeled and briefly described as follows: the negative controls receiving no butter fat; the controls receiving one-half of the full amount of butter fat; the controls receiving the full amount; and finally the test animals receiving either of the mineral oils along with the full amount of the butter fat. Vitamin A deficiency was gauged as follows: 1.—by growth, the animals being weighed twice a week; 2.—by eye condition, the eyes being examined carefully once a week; 3.—by vaginal smears; 4.—by necropsy findings. At the end of 75 days the animals were all killed and a thorough search for gross abnormalities was made. Particu-

CHART 3.



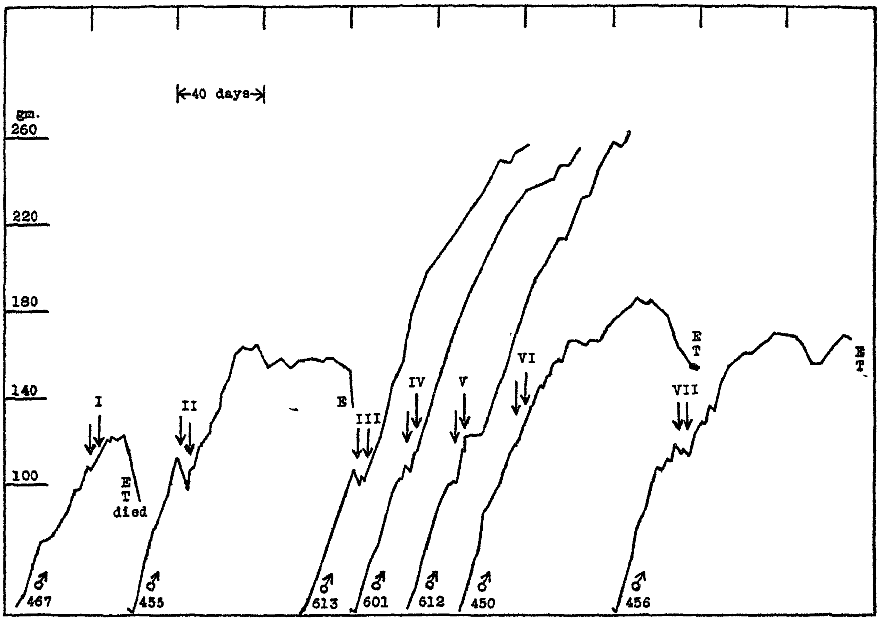
lar attention was paid to the occurrence of abscesses in the glands in the base of the tongue since such pathological conditions have been found in this laboratory both by Tyson and Smith (1929) and by ourselves to appear quite uniformly among animals reaching a certain degree of severe vitamin A exhaustion (cf. Sherman and Munsell, 1925). Two large series of experiments were carried out, one with 150 mgm. and a second with 100 mgm. of butter fat as the amount fed.

Discussion of Results

Inasmuch as some of our first experiments designed to furnish the test animals barely an adequate amount of butter fat were difficult of interpre-

tation, because all the growth curves, control and otherwise, fluctuated considerably, we decided in the present studies first to ascertain the effect

CHART 4.



EXPLANATION OF CHARTS 4 AND 5

The Roman numerals indicate the introduction of butter fat and mineral oil in daily dosages as follows:

I. No butter fat.
II. 50 mgm. of butter fat.
III. 100 mgm. of butter fat.
IV. 100 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 1 *separately.*
V. 100 mgm. of butter fat and 0.5 cc. of Mineral Oil No. 2 *separately.*
VI. 100 mgm. of butter fat *mixed with* 0.5 cc. Mineral Oil No. 1.
VII. 100 mgm. of butter fat *mixed with* 0.5 cc. Mineral Oil No. 2.

Letters adjacent to the ends of the curves represent necropsy findings:

E. Definite ophthalmia of varying severity.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.
K. Abscess formation in the kidney.
B. Bladder stones.

ADDITIONAL EXPLANATION OF CHART 5

(*See opposite page.*)

Since all graphs are arbitrarily drawn as though every rat survived the 75-day experimental period, it should be noted that all rats of Group I and one rat each of Groups II, VI and VII died within the 75 day period. Numbers used as coefficients in connection with the necropsy findings record the total separate cases in which a finding was made. The group average final weights are represented by a horizontal line marked with a circle.

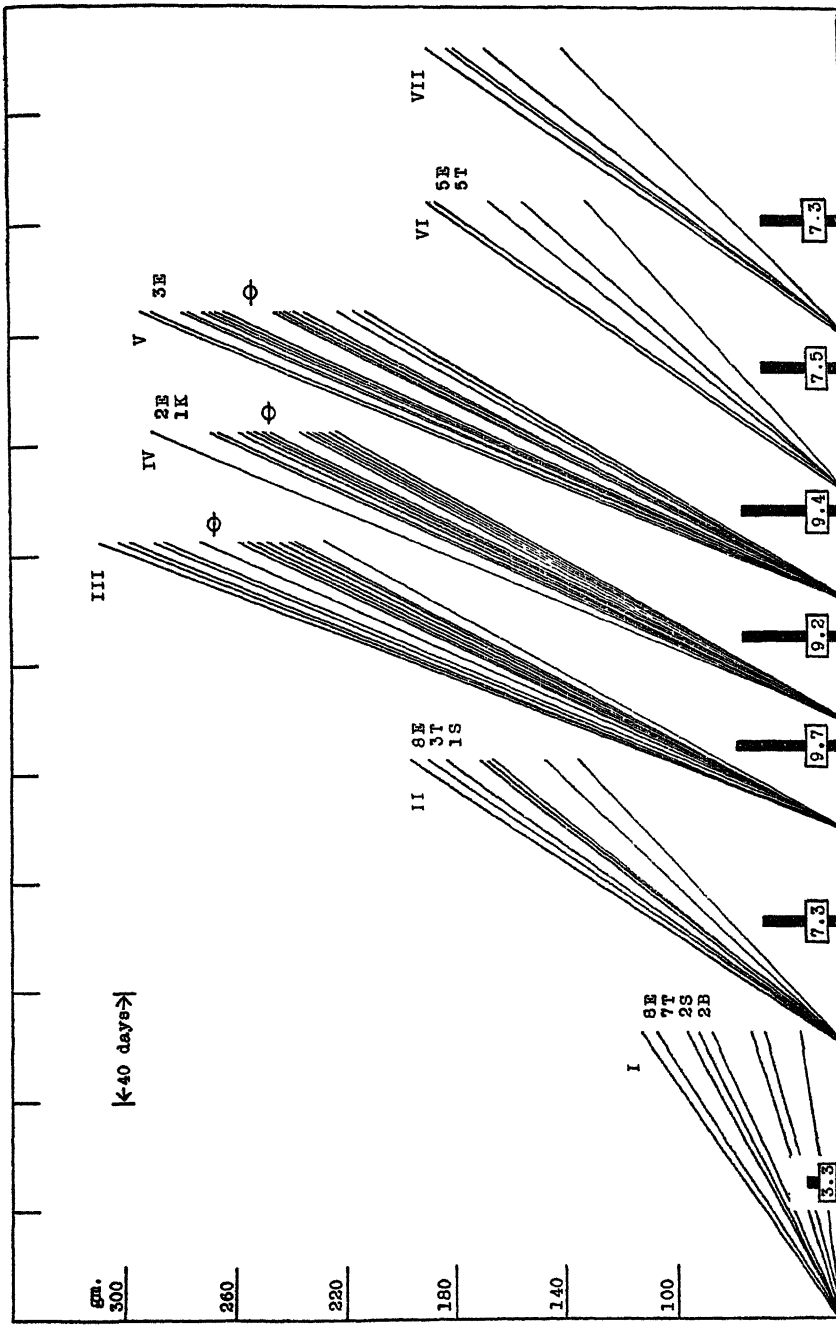

gm.
300
260
220
180
140
100
40 days
I
8E
7T
2S
2B
II
8E
3T
1S
III
IV
2E
1K
V
3E
VI
5E
5T
VII
3.3
7.3
9.7
9.2
9.4
7.5
7.3

CHART 5.

of feeding an adequate amount of vitamin A plus a 50 per cent safety margin. Eighteen males and eighteen females were employed in the experiment. Since the failure of the basal diet to maintain the animals had been repeatedly demonstrated, only two rats of each sex were used as negative controls. (Group I, see Charts 2 and 3.) The remaining 32 animals were divided into four groups each containing four males and four females. The daily individual doses of butter fat for these four groups were 75 mgm. for Group II, and 150 mgm. for Groups III, IV, and V. (See again Charts 2 and 3.) Each rat in Group IV received separately 0.5 cc. of Mineral Oil No. 1, and each rat in Group V, 0.5 cc. of Mineral Oil No. 2, according to the routine schedule already outlined.

The four rats of one sex on any one régime grouped quite well as to body weight attained at the end of the experiment. It will be seen in Chart 2 that the males receiving 150 mgm. of butter fat grew better than the males receiving only 75 mgm., thus confirming our assay findings that 100 mgm. of this butter fat were very close to the amount just necessary for adequate growth and for healing of eye lesions developed during the depletion period. It will be further observed that, whether they received mineral oil or not, there is no significant difference between the various 150 mgm. groups as to growth, eye condition or pathological changes as shown at necropsy. During the seventh ten-day period a daily examination of vaginal smears from all the female rats was made. Persistent cornification was revealed in all four of the rats receiving 75 mgm. of butter fat, but its absence was noted in all females receiving 150 mgm. of butter fat whether mineral oil was given additionally or not.

Since no perceptibly untoward results, as measured by the previously described indices, had developed in consequence of giving 0.5 cc. of mineral oil daily separate from the 150 mgm. dose of butter fat, we next investigated the effect of the feeding of 100 mgm. daily doses of butter fat. To detect appreciable differences now more likely to occur, only males were used and greater emphasis was placed on what has always appeared to be the most reliable index, *growth*. Eyes were examined and necropsies made, however, as before. A separate group of rats was used to determine once more (see Chart 1), simultaneously under identical conditions, the effect of administering the 0.5 cc. of mineral oil and the 100 mgm. of butter fat when *mixed together*. Charts 4 and 5 illustrate the findings of these undertakings. In line with former results, the rats securing their vitamin A already mixed with the mineral oil displayed marked depressions in growth. However, again (*cf.* Charts 2 and 3) there is exhibited little difference

between the rats receiving mineral oil separately and those not receiving it at all. In general the rats in these groups have attained about the same weight levels. Nevertheless, some instances of unhealed eye lesions and one case of kidney abscess were found among the animals receiving oil, and what is more significant, perhaps, the weight average of each oil group including fourteen to sixteen animals is somewhat lower than that of the control group. It follows from the statistical data given in Table II that

TABLE II

MEANS AND STANDARD DEVIATIONS (SIGMA) OF MEAN DIFFERENCES OF FINAL BODY WEIGHTS

Rat group	No. of animals	Final weight mean	Sigma of distribution	Sigma of the difference in means
		gm.	gm.	gm.
Mineral oil No. 1	14	247.0	19.0	
				8.8
Control	14	267.0	24.0	
				8.8
Mineral oil No. 2	16	253.0	24.3	

the chances that the mean weight of the controls is greater than the mean weight of the mineral oil animals are 99 out of 100 for the Mineral Oil No. 1 comparison, and 94 out of 100 for the Mineral Oil No. 2 comparison. It is fairly certain, then, that mineral oil slightly but definitely retards growth when given separately from the 100 mgm. dose of butter fat.

These observations indicate that, although mineral oil (both brands), when mixed directly with an otherwise adequate amount of butter fat, exerts a markedly harmful effect upon the vitamin A economy of the rat, the same amount of the same oil, given in doses separate but seriatim, beginning before and ending after the ingestion of the butter fat, causes only a slight depression of growth and only a questionable increase in ophthalmia and abscess formation. They indicate further that when a 50 per cent safety margin of the vitamin is present, no detectably adverse action occurs as judged additionally in this instance by vaginal smears. It is obvious from the foregoing experiments that although a vitamin A deficiency such as may be brought about *by mixing mineral oil with the butter fat* should be avoided, it does not necessarily follow that under the conditions of the *customary human therapeutic practice*, mineral oil is responsible for any such deficiency.

Summary

1. Mineral oil causes a considerable loss of vitamin A to the animal organism, if the mineral oil is *mixed with* the vitamin A (in the form of butter fat) prior to ingestion.

2. The administration of the mineral oil *separately* from the butter fat results in only a very slight diversion of vitamin A.

3. Moderate increase of the butter fat intake appears to protect the animal from any vitamin A deficiency when the mineral oil is given separately.

In conclusion, the writer wishes to acknowledge the helpful suggestions made by various members of the Department, especially those made by Professor Lafayette B. Mendel.

Addendum

Several months after this paper was submitted for publication, there appeared a contribution by Rowntree (1931) on the "The Effect of the Use of Mineral Oil Upon the Absorption of Vitamin A." The results obtained in many respects comparable to those reported here will be commented upon in a later communication.

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VITAMIN A DEFICIENCY IN THE ALBINO MOUSE*

By

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THAT the epithelium of the various structures of the body undergoes extensive changes when there is a deficiency of vitamin A in the diet, has been shown in the case of man by Wilson and DuBois (14), and for the rat and the guinea pig by the work of Wolbach and Howe (15, 16). In man and the rat, the normal epithelium of the respiratory tract, the alimentary tract, the genito-urinary tract, and the eye and paraocular glands is desquamated and is replaced by keratinized epithelium. The guinea pig shows similar changes, although the eye and paraocular glands are usually not affected.

Wolbach and Howe state that the keratinization can take place in the absence of infection and that, if infection does occur, it is secondary to the changes in the epithelium which, they think, are due directly to the lack of vitamin A. On the other hand, Tyson and Smith (13) have pointed out that infection is always present, even in the earliest stages of the deficiency and, in the later stages, always dominates the picture.

Nelson and Lamb (9) fed rabbits on a synthetic diet to which alfalfa, extracted with alcohol, had been added. These animals developed ophthalmia which was relieved by adding butter fat to the diet. Steenbock, Nelson, and Hart (12), in their studies on calcium assimilation, found that dogs developed ophthalmia on the diet used. They were able to cure this ophthalmia by adding vitamin A to the diet. Beach (1) has reported that fowls fed on a diet deficient in vitamin A suffer from ophthalmia, nasal discharge, lesions of the mucosa of the mouth, pharynx, and esophagus; and, in the later stages, become weak and emaciated.

Beard (2), in his studies on the nutrition of the white mouse, was unable to produce the typical symptoms of xerophthalmia in white mice fed on a diet deficient in vitamin A, even though the other symptoms of A deficiency were present, *i.e.*, arrest and decline of growth, and roughened fur. Spinka (11), while studying the effect of ultra-violet rays on the accessory food substances, found that his mice developed a typical xerophthalmia

* This work has been supported by a grant from the Commonwealth Fund.

and died in about 50 days. Fujimaki (4), in studying the formation of urinary and bile duct calculi in animals fed on various experimental diets, found that mice fed on a diet deficient in vitamin A, developed xerophthalmia and died in about 25 days.

Recently Pomerene and Beard (10) have made a further study of this question and, contrary to Beard's first results, they have been able to produce xerophthalmia in the mouse in 50 to 60 days. In these experiments they were unable to produce xerophthalmia on any diet that contained hydrogenated vegetable fat[1] unless the fat was aerated before use. From these results they came to the conclusion that the fat used contains enough A to protect mice from the typical eye symptoms, although it does not contain sufficient A to promote good growth. They attribute their failure to produce xerophthalmia in earlier experiments to the fact that their diet contained 24 per cent hydrogenated vegetable fat[1]. It is well known, from the results of many investigators, that the presence of this fat in a diet otherwise deficient in vitamin A will not prevent xerophthalmia in rats. Thus Pomerene and Beard conclude that the requirements of rats and mice for the anti-xerophthalmic factor are different, rats requiring vitamin A at a higher level than do mice.

We have made a study of A-deficiency in white mice to determine whether or not they undergo the extensive changes of the epithelium throughout the body which have been described by Wolbach and Howe for the rat and guinea pig. In this study we have used 50 mice, both males and females being included in the series. They were placed on a diet when they had reached a weight of 9 to 12 grams. In all cases litter mate brothers or sisters were used as controls. All except the first series, which consisted of twelve mice, were kept in separate wire cages with a mesh bottom. Those of the first series were kept in two groups of six. The following experimental diet was used:

	Per cent
Casein (purified)	31
Dextrin	35
Hydrogenated veg. fat[1]	22
Salts (Osborne and Mendel)	7
Yeast (Harris)	5

The control diet was the same, except that cod liver oil (Harris) was added at a 3 per cent level and the percentage of fat[1] lowered to 19.

The length of time required for the mice to develop xerophthalmia

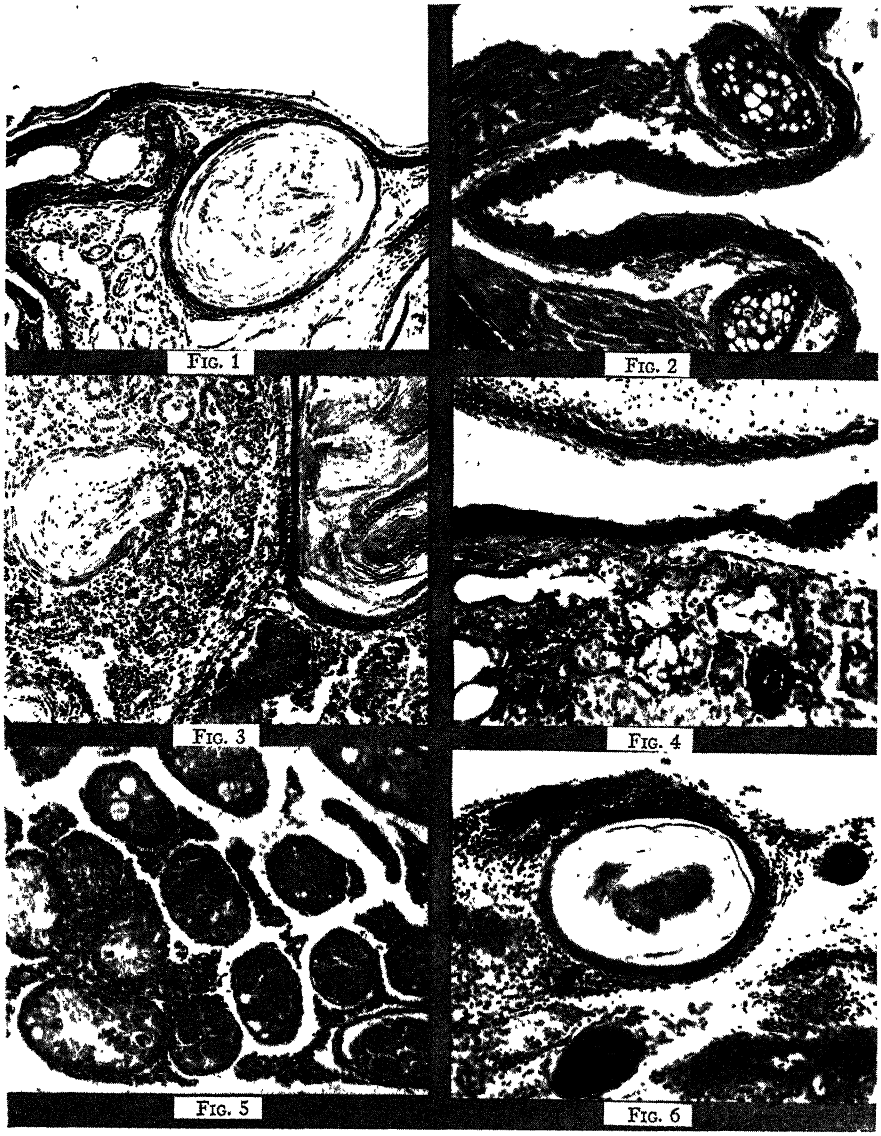
[1] Crisco.

varied greatly. In some cases they showed the typical eye symptoms in 35 days, but in a majority of the cases a much longer time was required (90 to 120 days). The experimental animals almost invariably showed eye symptoms before they began to lose weight.

The animals were killed at various stages of the deficiency in order to get as comprehensive a picture of the tissue changes as possible. Those animals which were allowed to develop the most advanced condition before being sacrificed had assumed a roughened coat and a humped posture which is the characteristic appearance of rats fed on an A-deficient diet. The eyes were encrusted with a granular appearing exudate which, in most cases, was not hemorrhagic as is usually found in rats. Placques of desquamated cells were found on the cornea. The animals became extremely weak and often were scarcely able to support themselves. At this stage of the deficiency, they usually weighed 13 to 15 grams. Almost invariably they exhibited respiratory distress. Diarrhea was also a common symptom in animals which were allowed to reach the later stages. The food intake in later stages of the disease was extremely low.

The gross postmortem changes were very marked. There was a complete disappearance of the body fat. The thymus was markedly atrophic in practically every animal. The salivary glands were usually about one-half normal in size. In many cases the lungs showed large hemorrhagic spots. The spleen was usually very dark in color and extremely small. The pelvis of the kidney was sometimes filled with a granular opaque mass, which was later found to be made up of desquamated cells. In one case a large renal calculus was found in the pelvis of the kidney. In a large number of animals the bladder wall was thickened and the bladder was filled with a mass similar to that found in the pelvis. The seminal vesicles were usually extremely distended and filled with similar masses. The testes were small soft, and usually very watery when cut.

The tissues from 16 animals were studied histologically. They gave a picture very similar to that described for the rat and guinea pig by Wolbach and Howe. They found that the various types of epithelium of the body organs were replaced by keratinized cells which usually began to grow in small clumps beneath the normal cells. These "foci" of cells grew by peripheral extension beneath the apparently normal epithelium. The original epithelium seemed able to survive for a considerable length of time, even though it was separated from its connective tissue support by the keratinized cells. Although we have observed these "foci" of cells in mouse tissues, the more common finding was an orderly layer of keratin-



DESCRIPTION OF PLATE

FIG. 1. Photomicrograph of a cross-section of the nares of mouse no. 86 which had been on experimental diet for 100 days. X 150.

FIG. 2. Photomicrograph of a cross-section of the trachea of mouse no. 84 which had been on experimental diet for 100 days. X 150.

FIG. 3. Photomicrograph of a cross-section of the submaxillary gland of mouse no. 84 which had been on the experimental diet for 100 days. X 150.

ized cells which seemed to begin at only one point and grow in all directions. We have never noticed several foci in any one section.

The first changes usually appeared in the respiratory tract (Figure 1), as described by Wolbach and Howe for the rat, but were closely followed by the symptoms of xerophthalmia. All stages of keratinization and desquamation were observed. In no case was there a purulent exudate in the nares. In some few cases the histological sections have shown some evidence of infection. In the nares, trachea (Figure 2), and bronchi there was extensive keratinization of the epithelium, accompanied by marked desquamation. In the lungs, bronchiectases were frequently found. They were probably caused by the occlusion of the bronchi by desquamated cells.

In the stomach and intestine no changes were evident. An extremely large amount of desquamation was observed in the esophagus. A similar desquamation, though in a much smaller degree, was observed in normal control mice. No constant changes were found in the liver. The submaxillary glands of our mice did not seem to be affected as early or to the same extent as Wolbach and Howe (15) found to be the case in the rat. We have not been able to demonstrate any change in the secreting epithelium except perhaps a slight atrophy. In the ducts, however, in the later stages of A-deficiency, we have observed all stages of the typical changes from a beginning of keratinization to a complete replacement of the normal epithelium by the keratinized type (Figure 3). In the latter case the ducts were completely filled with desquamated material. We have not observed any degenerative changes in the accessory salivary glands at the base of the tongue.

Extensive changes were noticed in the bladder and the pelvis of the kidney, the normal epithelium being replaced by keratinized epithelium. The bladder in many cases was completely filled with desquamated cells, while the pelvis of the kidney showed desquamation to a lesser degree. A leucocytic infiltration was often seen in the latter. In the female genital tract there were no noticeable changes. The ovaries, fallopian tubes, uterus and uterine glands appeared normal.

The testes showed various stages of degenerative changes. The first

FIG. 4. Photomicrograph of a cross-section of the cornea and the eye-lid of mouse no. 86 which had been on experimental diet for 100 days. X 150.

FIG. 5. Photomicrograph of a section of the testis of mouse no. 41 which had been on experimental diet for 119 days. X 150.

FIG. 6. Photomicrograph of a section of the prostate gland of mouse no. 48 which had been on the experimental diet for 79 days. X 150.

change noted was some desquamation of the germinal epithelium. As degeneration progressed, we found large amounts of a structureless material, which contained many clear spaces or vacuoles. It is probable that this structureless material was made up of masses of giant cells which had degenerated to such an extent that all cell structure was lost (Figure 5). That such giant cells are formed in the degenerative process, has been shown by Mason (7 and 8) in his work on rats, fed diets deficient in either vitamin A or E. In our experiments only the primary germ cells and the sustentacular cells were left in the tubules in the later stages. The tubules were shrunken and evidently had been surrounded by a watery fluid. At autopsy the testes were always soft and watery. No changes were noticed in the interstitial cells.

The prostate and seminal vesicles were, in most cases, greatly distended with desquamated masses (Figure 6). Histological examination showed that the original epithelium was completely replaced by keratinized epithelium. The lumina of the tubules were completely filled with sheets of desquamated cells, and were so distended that they appeared cyst-like. The connective tissue between the tubules was greatly increased. In many instances there was a down-growth of the keratinized epithelium into the subjacent connective tissue. These changes resemble those described by Wolbach and Howe for the rat and guinea pig. No changes were observed in the epithelium of the epididymis.

The adrenal and thyroid appeared normal. Although there was a complete or nearly complete atrophy of the thymus, histological examination of the persisting tissue revealed no constant changes.

The eyes and eyelids from fifteen mice have been studied histologically. Compared with other organs, the changes in the eyes were slight, although at autopsy a severe granular exudate was usually seen. The most outstanding feature was the keratinization and desquamation of the epithelium of the palpebral conjunctiva which, in some cases, was quite severe. The Meibomian glands showed various degrees of atrophy and vacuolization. The changes in the cornea were for the most part slight. In some cases there was a slight keratinization, accompanied by some desquamation. In a few instances a slight infiltration of leucocytes into the connective tissue of the cornea was noticed. Bowman's membrane was usually intact.

In one case the cornea had increased in thickness until it was 4 or 5 times as thick as is usually found. There was an extensive keratinization of the epithelium of both the cornea and the conjunctiva, accompanied by a large amount of desquamation. In the cornea there was a great increase in

the vascularity of the substantia propria, which was also very edematous. There was also an extensive infiltration of leucocytes. Bowman's membrane had disappeared and there was a down-growth of the keratinized cells into the connective tissue below. A considerable number of polymorphonuclear leucocytes were found in the anterior chamber of the eye. The animal referred to above was the only one in which an extreme eye condition was noted.

As shown by Wolbach and Howe for the rat, and we have found a similar condition in the mouse, the changes in the epithelium of the eye occur later than those of the respiratory tract, so that it would seem that the epithelium of the eye is less susceptible to the A-deficiency than that of some other organs of the body. A striking difference was found between the exudate exhibited by the eye of the mouse and that of the rat. In the mouse, in our experience, the exudate is usually not hemorrhagic, but is light in color and granular, while in the rat the exudate is very hemorrhagic.

Discussion

In the diet used in these experiments, the hydrogenated vegetable fat was the only source of vitamin E. It was supplied at a 22 per cent level. It has been shown by Kennedy and Palmer (6) that, when this fat is incorporated in the diet at a 15 per cent level, successful reproduction is secured. Evans and Burr (3) secured "cures" in sterile female rats by feeding it at a 22 per cent level. From these observations it seems evident that the diet we used contained a sufficient amount of vitamin E, and therefore that the severe testicular degeneration observed was due to the lack of vitamin A.

We have not found marked evidence of infection, even in the later stages of the deficiency. In many cases we have observed a complete replacement of the normal epithelium by the keratinized type, with no sign of infection present. In general, the histopathology found has been very similar to that described by Wolbach and Howe for the rat and guinea pig. The diet used was also deficient in vitamins C and D. However, it has been shown by the work of Goldblatt and Benischek (5) that the lack of these vitamins plays no part in the metaplastic changes, so it is evident that the keratinization observed is due directly to the lack of vitamin A in the diet. Since our experimental diet contained hydrogenated vegetable fat at practically the same level as used by Beard, we are at a loss to explain why our animals developed xerophthalmia, while his did not.

Summary and Conclusions

In both male and female mice, placed on an A-deficient diet at about 25 days of age, the normal epithelium of the various structures was replaced by keratinized epithelium. The mice showed evidence of xerophthalmia in 25 to 120 days. However, the severity of the eye symptoms was slight as compared with the changes undergone by other structures of the body. We have repeatedly observed metaplastic changes in the respiratory tract before any eye symptoms were present. The male mice suffered testicular degeneration similar to that described by Mason for rats, fed on both A- and E-deficient diets.

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THE VITAMIN VALUE OF COD LIVER MEAL

By

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(From the Research Laboratories, The E. L. Patch Company, Boston.)

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COD LIVER MEAL is obtained as a by-product in the cod liver oil industry. In the manufacture of cod liver oil by direct stem process, a large portion of the oil and water-soluble proteins are removed from the liver tissues. The remaining liver tissue, after it is dried and ground, is sold as "cod liver meal." The physical appearance of cod liver meal varies greatly due primarily to the length of time which elapses between cooking the livers and the subsequent drying. When the liver residue is dried very promptly after the oil is extracted, a good quality of cod liver meal is obtained. On the other hand, when the liver residue is allowed to remain in a moist condition several months previous to drying, the resulting liver meal is a dark color and has a pronounced odor.

Considered from a nutritive standpoint, cod liver meal contains about 40 per cent of protein and nearly as much fat. In view of the relatively large amount of residual fat in cod liver meal, it has been frequently suggested that it could be used in poultry rations instead of cod liver oil as a source of vitamins. The present investigation was undertaken to secure data concerning the vitamin value of commerical cod liver meal.

Four lots of commercial cod liver meal were obtained on the open market. They were obtained from different sources and were apparently free from fish scraps. Three of the meals, 1, 2, and 4, were reported to be of Norwegian origin. The fourth meal, 3, was supposed to have been produced

Table I

Results of Analyses of Cod Liver Meals

Per Cent	Meal 1 (test 1)	Meal 2 (test 2)	Meal 3 (test 3)	Meal 4 (test 4)
Moisture	5.47	5.27	6.15	7.25
Ash	2.15	2.53	3.87	3.28
Protein	46.08	37.65	38.04	48.25
Fat	33.16	43.38	34.38	27.15
Carbohydrate	13.14	11.17	17.56	14.09
Calcium	0.09	0.02	0.21	0.18
Phosphorus	0.41	0.54	0.73	0.72

in Nova Scotia and Newfoundland. Samples of the meals were analyzed by the Official Methods of Agricultural Chemists (1) and were found to have the composition shown in Table I.

As will be noted from the results obtained, the chemical composition of the four meals varied widely. The greatest variation was in the protein and residual fat content. Lesser, but significant differences occurred in the calcium and phosphorus content of the meals.

Experimental Procedure

Rhode Island Red baby chicks, were housed in an all-metal type, electrically-heated brooder. The brooder was situated in the northern portion of the laboratory, where no direct sunlight was available. The chicks received an all-mash ration unsupplemented by grit or additional sources of lime. The composition of the all-mash ration was as follows:

Composition of Experimental Ration

	Per cent
Yellow corn meal, No. 2	30.0
Standard wheat bran	15.0
Wheat flour middlings	15.0
Ground oat groats	15.0
Dried buttermilk	8.0
Alfalfa leaf meal	5.0
Steamed bone meal	4.0
Fish meal (55% protein)	3.5
Meat scraps	3.5
Salt	1.0

The chicks were divided into eight groups (pens) of twenty each. Each group was allowed six square feet of space which was ample since four chicks were removed from each pen on the 21st, 35th, and 56th day of the test. The four experimental pens received the basal ration plus 2 per cent of cod liver meals 1, 2, 3, and 4 respectively, incorporated in the ration.

The tests of the cod liver meals were made at four different periods. In each instance, four pens (controls) were fed cod liver oil at four different levels, namely: 0.13, 0.25, 0.50, and 1.00 per cent of the ration. For the purpose of this discussion, the pen receiving cod liver oil which most nearly duplicated the results obtained with the cod liver meal under consideration has been selected as the "control" pen. Thus in test 1 the pen receiving 0.50 per cent cod liver oil, in test 2 the pen receiving 0.50 per cent cod liver oil, in test 3 the pen receiving 0.25 per cent cod liver oil, and in test 4 the pen receiving 0.13 per cent cod liver oil have been selected as "control" pens.

The chicks and food were weighed weekly as individual pens. The experi-

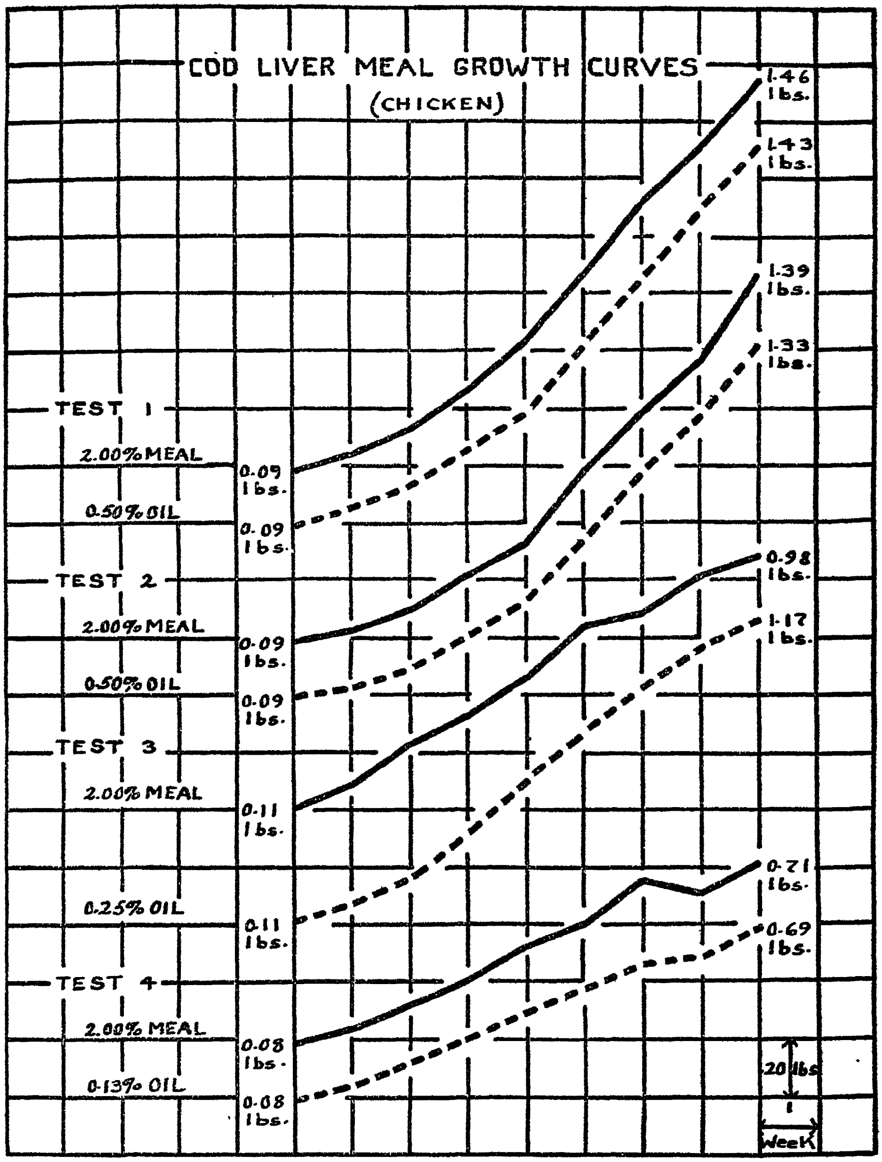

CHART 1.

ment was of eight weeks' duration. The value of the different cod liver meals as sources of vitamins has been judged by the general physical appearance of the birds, their rate of growth, mortality and their bone development.

Growth of Chicks

The weights of the chicks at the end of each week were averaged and reduced to a per chick basis. These results have been plotted in Chart 1.

The growth of the chicks receiving 2 per cent of meals 1 and 2 corresponded with that of the chicks receiving 0.50 per cent of cod liver oil. The rate of growth of chicks receiving 2 per cent of cod liver meals 3 and 4 was considerably less. When compared with duplicate pens receiving cod liver oil, it was found that the rate of growth obtained for cod liver meal 3 was equivalent to that obtained with 0.25 per cent cod liver oil and the rate of growth of the pen receiving cod liver meal 4 was equivalent to that of the pen receiving 0.13 per cent cod liver oil. The variation in rate of growth of the chicks which received the different meals apparently cannot be correlated with either the protein or the fat content of the meals. For instance, meals 1 and 2, which produced a similar growth curve, differed by approximately 10 per cent in both protein and fat content. Furthermore, the rate of growth obtained with meal 4, which had the highest percentage of protein and nearly as much fat as meal 1, was very unsatisfactory and only about half that obtained with meal 1.

Mortality

In recording the mortality of chicks during the four cod liver meal experiments, no record was made of the chicks removed at the 21st, 35th, and 56th day for examination of the tibiae. The following table reports the date and number of mortalities which occurred during the experiments under discussion.

Table II

Mortality Record

Test 1	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Total
2.00% Meal........	2	—	—	—	—	—	—	—	2
0.50% C.L.O........	—	—	—	—	—	—	—	—	—
Test 2									
2.00% Meal........	—	—	—	—	—	—	—	—	—
0.50% C.L.O........	—	—	—	—	—	—	—	—	—
Test 3									
2.00% Meal........	—	—	—	—	—	—	—	—	—
0.25% C.L.O........	1	—	—	—	—	—	—	—	1
Test 4									
2.00% Meal........	—	1	—	—	—	—	1	4	6
0.13% C.L.O........	1	—	—	—	—	—	—	2	3

From the above table, it will be noted that the mortality was twice as large for the cod liver meal as for the cod liver oil chicks. This result agrees with that obtained by Stuart (2) who reports twice the mortality for the chicks fed 2 per cent cod liver meal in the basal ration as for a comparable pen fed cod liver oil as a vitamin supplement. Somewhat similar mortality was reported by Mussehl (3) and co-workers who lost 47.5 per cent of the birds fed cod liver meal as a supplement to their basal ration, 62.5 per cent of the birds receiving the basal ration unsupplemented, and only 5.0 per cent of the birds which received cod liver oil as a supplement to the basal ration.

Bone Development

While space does not permit the inclusion of photographs of all the tibiae removed from chicks during this investigation, it is felt that it will

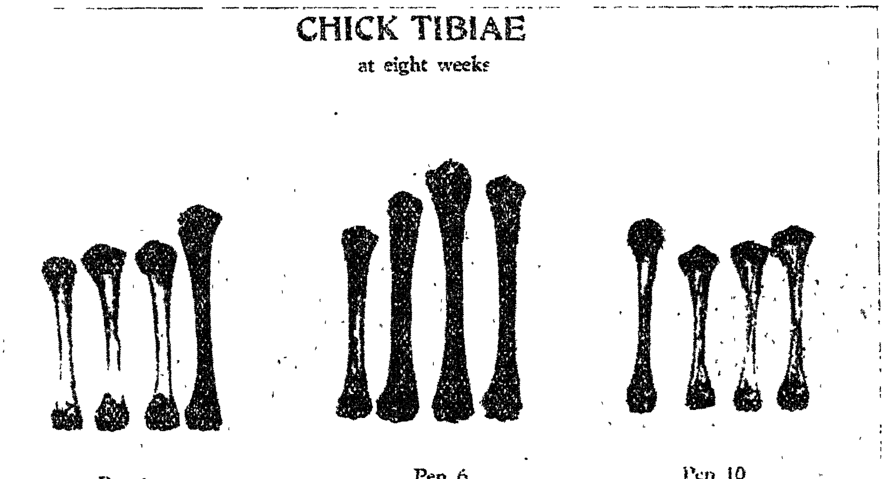

Fig. 1.

assist the reader better to visualize the extent of bone development obtained if a photograph of one series of tibiae is presented. Accordingly, the accompanying photograph (Figure 1) includes, tibiae from the negative control pen (pen 1) which received the basal ration unsupplemented, tibiae from the positive control pen (pen 6) which received the basal ration supplemented with 0.13 per cent cod liver oil, and tibiae from the experimental pen (pen 10) which received the basal ration supplemented with 2.00 per cent cod liver meal 4. The differences in development of the tibiae represented are very evident to the eye.

At the termination of the third, fifth, and eighth weeks, four representative chicks from each pen were killed. Both legs were dissected and freed of adhering tissue. For the ash determination, the left tibiae were dried for 48 hours at 96° C., crushed and extracted with hot alcohol (95 per cent) for 24 hours, weighed and ashed in an electric muffle furnace. The data obtained are recorded in Table III.

TABLE III

AVERAGE PERCENTAGE OF ASH IN THE LEFT TIBIAE (FAT FREE)

Test 1	At Three Weeks	At Five Weeks	At Eight Weeks
2.00% Meal	44.78	43.32	48.43
0.50% C.L.O.	42.02	49.27	48.55
Test 2			
2.00% Meal	44.47	44.98	46.63
0.50% C.L.O.	44.20	47.35	47.42
Test 3			
2.00% Meal	41.41	46.27	—*
0.25% C.L.O.	43.35	39.63	42.17
Test 4			
2.00 Meal	38.56	37.60	39.82
0.13% C.L.O.	37.49	36.92	40.09

* The determination of the ash content of the dried, extracted tibiae from the experimental chicks in test 3 at eight weeks of age, gave a value of 26.51 per cent. Obviously, this value is unusually low, but by the time it was obtained the chicks were too old to be used for a redetermination of the ash content of the tibiae. The histological report of the extent of calcification of the tibiae is more or less in accord with an ash content of 26.51 per cent, *i.e.*, "wide epiphyseal cartilage, abnormal marrow, space filled with cancellous osseous tissue."

The percentage of bone ash of those chicks receiving meal 4 (test 4) indicated severe rickets and corresponded closely to the oil control pen receiving only 0.13 per cent cod liver oil. Meal 3 (test 3) gave slightly better results, agreeing with the control pen receiving 0.25 per cent cod liver oil, although there was a decided drop in the percentage of ash between the fifth and eighth weeks. The percentages of ash in the tibiae from chicks which received meals 1 and 2 (tests 1 and 2) were fairly comparable and approximately that of the control pens which received 0.50 per cent cod liver oil. In general, the ash results were in accord with the growth curves.

CALCIFICATION

To obtain a more complete knowledge of the antirachitic value of the cod liver meals, the extent of calcification was determined by a modification

of the line-test method as described by McCollum (4) and his co-workers. The right tibiae, taken from test chicks on the 21st, 35th, and 56th day of the test, were freed of adhering tissue and immersed in 10 per cent formalin for 24 hours. They were then washed and longitudinal sections were cut from the proximal end. The sections were treated with 2 per cent silver nitrate and intensified under bright light until the calcified area became dark brown, indicating the degree of calcification. The results are recorded below.

TABLE IV

DEGREE OF CALCIFICATION

Basal Ration supplemented with	At Three Weeks	At Five Weeks	At Eight Weeks
	Test 1		
2.00% Meal	Normal	Normal	Fair
0.50% C.L.O.	Abnormal	Normal	Normal
	Test 2		
2.00% Meal	Normal	Normal	Normal
0.50% C.L.O.	Normal	Normal	Normal
	Test 3		
2.00% Meal	Fair	Poor	Very poor
0.25% C.L.O.	Abnormal	Abnormal	Poor
	Test 4		
2.00% Meal	Poor	Poor	Very poor
0.13% C.L.O.	Poor	Poor	Very poor

The results show that meal 2 (test 2) was superior to all the other meals for promoting calcification. Apparently cod liver meal 2, when used in conjunction with the relatively high vitamin ration employed in this study contained sufficient antirachitic vitamin to protect chicks from rickets for a period of eight weeks. Meals 3 and 4 (tests 3 and 4) possessed the poorest calcifying properties and afforded little if any protection against rickets, while meal 1 (test 1) assumed a midway position, affording protection for only about five weeks.

DISCUSSION

From the foregoing data it will be noted that the four samples of cod liver meal differed significantly in their vitamin value. Judging from all the results obtained, namely; rate of growth, tibial percentage of ash, and deposition of calcium, meal 2, having an antirachitic value equivalent to 0.50 per cent cod liver oil, was far superior to meals 3 and 4. Perhaps one would have anticipated this result since cod liver meal 2 contained the

highest percentage of fat, but Bethke (5) and co-workers report that, "The antirachitic variation is not proportional to the residual fat content of the livers." This conclusion was confirmed in the results of the test with cod liver meals 1 and 3 (tests 1 and 3) which have practically the same fat content, but which produced decidedly different growth and bone development.

Meals 3 and 4 (tests 3 and 4) were decidedly the poorest. The growth and physical condition of the chicks were extremely poor. They exhibited bad cases of leg weakness, staggering gait, lack of control and spasms of trembling, all of which, according to Cruickshank (6) and co-workers, may have been indications of lack of vitamin A.

Thus it is apparent that the vitamin content of cod liver meal is extremely variable and the meal may be of little if any value as a source of vitamin D. This conclusion is in accord with the findings of Bethke (5) and co-workers who report that "it would seem unwise to use the liver meal as an antirachitic substitute for a good grade of cod liver oil in either poultry or livestock production." The results of this investigation are also in harmony with those reported by Musselhl, *et al;* (3) who conclude that "cod liver meal made entirely from dried ground liver tissue contains some vitamin D, but not as much as is associated with an equal amount of fat in cod liver oil." These results are not in accord with those reported by Stuart (2) who states "cod liver oil and cod liver meal were quite comparable in relation to growth." Cruickshank, *et al*; (6) have also studied the vitamin A value of cod liver meal but their findings are not in agreement with those of Stuart for they report 10 per cent of cod liver meal was not sufficient to protect chickens against vitamin A deficiency.

Summary

Samples of cod liver meals were obtained on the open market. These were added to a typical poultry ration. Pens of twenty Rhode Island Red baby chicks were used for the test. Control pens received cod liver oil incorporated in the basal ration. The experimental period was of eight weeks duration and test birds were removed from each pen at the end of the third, fifth, and eighth week. These were killed, and the tibiae removed and examined to determine the extent of calcification. It was found that the best cod liver meals produced about the same results when 2 per cent was added to the basal ration as when one-half of one per cent of cod liver oil was added. The poorer meals were found to possess little if any vitamin value.

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A TENTATIVE METHOD OF ASSAYING FOODS FOR VITAMIN G*

By

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RECOGNITION of the fact that many of the effects formerly ascribed to vitamin B as a single factor were due to one or more associated factors has made necessary further study of assay methods. The early work on the differentiation of vitamins B and G (3) clearly shows that vitamin G has growth-promoting properties.

Methods for quantitatively comparing foods as sources of other growth-promoting vitamins have been made dependent upon the growth responses of suitable laboratory animals to measured amounts of a test food when fed as the sole source of the vitamin in question. These methods require a basal diet that is free from the vitamin to be tested. In the application of this method to vitamin G determination, a difficulty is encountered in the fact that no convenient food has been found that will supply to the basal diet an adequate amount of vitamin B without admixture of vitamin G. Consequently it has been necessary to prepare extracts of a food rich in vitamin B and low in vitamin G using a solvent which removes only minimum amounts of vitamin G. The preparation of such extracts involves much time and expense and many laboratories are not in a position either to prepare or to purchase them.

Goldberger (4) stated that, in preparing vitamin B concentrates, maize might serve as a better initial source than yeast since it was rich in vitamin B and relatively poor in the associated thermostable factor (vitamin G). Furthermore, in the summary of work on vitamin B in wheat and corn, Hunt (5) states that Croll and Mendel found 20 to 30 per cent of corn an adequate source of vitamin B.

It was the purpose of the present investigation to ascertain if, in the assay of vitamin G, white corn could be used to supply the necessary vitamin B.

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* Read before the meeting of the American Chemical Society at Atlanta, Georgia, April 8–11, 1930.

Experimental

White Corn as a source of vitamin B relatively free from vitamin G. The basal diet 107 of Sherman and Spohn (10) composed of purified casein, 18 per cent; starch, 68 per cent; butter fat, 8 per cent; cod liver oil, 2 per cent; and Osborne and Mendel salts, 4 per cent, contains no detectable amounts of vitamin B or vitamin G.

In order to test the adequacy of white corn as a source of vitamin B and also to ascertain the relative amount of vitamin G present, six modifications of this diet were prepared. In the first three, corn replaced the starch in the basal diet to the extent of 10, 20, and 30 per cent respectively, and was the only source of vitamins B and G. For convenience these diets are designated as 107–C10, 107–C20, and 107–C30. The other three diets were similar to these except that each contained 10 per cent of autoclaved yeast in place of an equivalent weight of starch. The yeast was autoclaved by placing it in petri dishes in layers $\frac{1}{4}$ inch thick and heating in an autoclave for 4 hours at 15 pounds pressure. Many tests carried out in this laboratory have shown that this procedure destroys any detectable amounts of vitamin B in the yeast. Thus in these three diets designated respectively as 107G–C10, 107G–C20, and 107G–C30, the corn continued to remain the sole source of vitamin B, while vitamin G was adequately supplied by the autoclaved yeast with corn as a second possible source.

Six groups of 28-day old rats were then given one each of these diets for a period of eight weeks. Chart 1 shows the results obtained with diets 107G–C10, 107G–C20, and 107G–C30. It is evident that neither 10 nor 20 per cent of white corn supplies an amount of vitamin B sufficient for normal growth under the conditions of this test, since 10 per cent did not make it possible for the rats to maintain their original weight and 20 per cent supported a gain of only 59.5 grams during the 10 weeks. However, 30 per cent of corn supplies sufficient vitamin B for normal growth during this period, since the rats on diet 107G–C30 gained 101.5 grams in the 8 weeks of the test. The intake of corn averaged 0.29 grams per rat per day on diet 107G–C10, 1.18 grams on diet 107G–C20, and 2.5 grams on diet 107G–C30.

Chart 2 gives the results with diets 107–C10, 107–C20, and 107–C30. From these results it can be seen that white corn contains very little vitamin G since the average gain in weight of the group of rats on diet 107–C30 was only 7 grams while on diets 107–C10 and 107–C20 the rats lost weight. That vitamin G and not vitamin B was the first limiting factor for growth is shown by comparing the average daily corn intake of the two groups of animals portrayed in Charts 1 and 2. In Chart 1 it will be noted

that 0.29 and 1.18 grams of corn per rat per day provided a source of vitamin B which was responsible for gains in weight of −4.5 and 59.5 grams respectively. In Chart 2, 0.27 grams and 1.21 grams of corn supplied both vitamins B and G and the respective gains in weight were −13.0 grams and 7.0 grams. In other words a relatively smaller amount of corn is required to furnish an amount of vitamin B for a given gain in weight than is required to furnish vitamin G sufficient for the same gain in weight.

Other tests were then carried out in order to further substantiate the

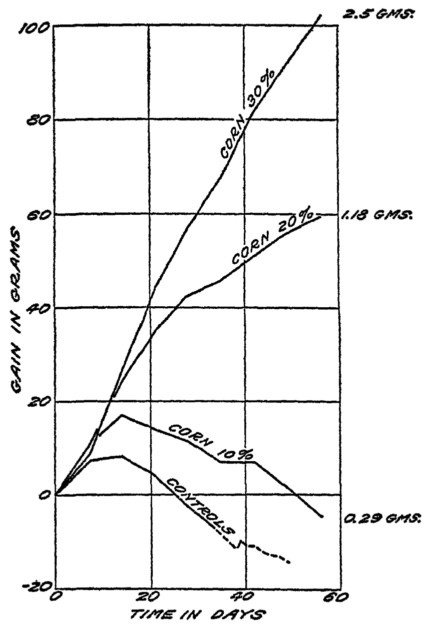

CHART 1. Curves showing average changes in weight of groups of rats fed a diet in which white corn was the sole source of vitamin B. The per cent of corn is indicated on the respective curves and the average intake of corn in grams per rat 6 times per week is given at the end of the curve.

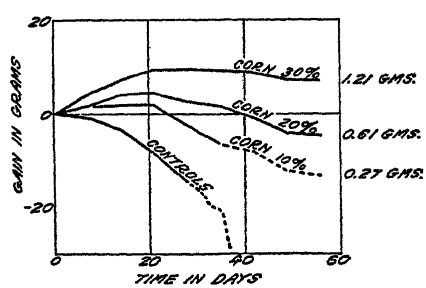

CHART 2. Curves showing average changes in weight of groups of rats fed a diet in which white corn was the sole source of vitamin B and vitamin G. The per cent of corn is given on the respective curves and the actual intake of corn in grams per rat six times per week is given at the end of each curve.

evidence that corn contains very little vitamin G. The results obtained by Mitchell (6) and later by Goldberger (4) using alcohol extracts of white corn as a source of the antineuritic vitamin indicated that vitamin B is readily soluble in alcohol of all concentrations up to 80 per cent by weight, and that vitamin G is decreasingly soluble with increasing concentration. Later, the work of Sandels (8) confirmed this impression. Accordingly, an

alcohol extract of white corn should contain the vitamin B originally present without admixture of significant amounts of vitamin G.

An 80 per cent (by weight) alcohol extract of white corn was prepared, evaporated down on cornstarch and tested for its vitamin B and G content. It was first tested for vitamin B by feeding at levels of 2, 3, and 5 per cent[1] replacing an equivalent weight of starch in diet 107G (diet 107 with 10 per cent autoclaved yeast in place of an equal weight of starch). The results of

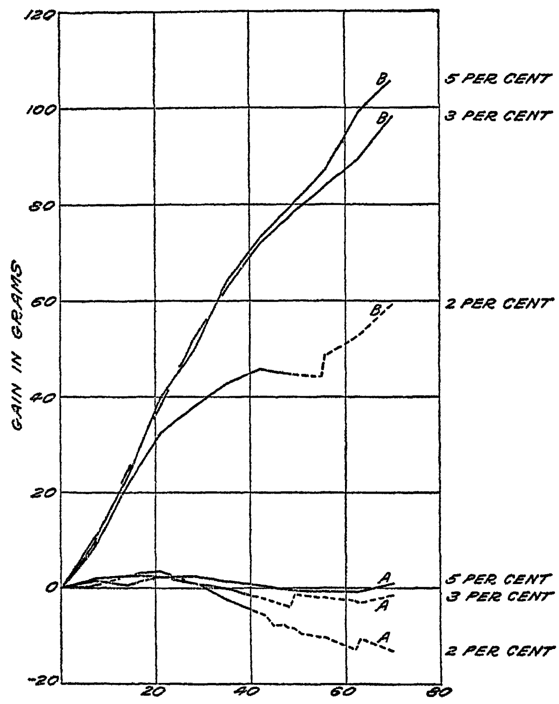

CHART 3. Curves showing change in weight of six groups of rats. Those designated by "A" received an 80 per cent (by weight) alcohol extract of white corn as the sole source of vitamins B and G. Those designated by "B" received the extract as the sole source of vitamin B. The percentage of extract included in the diet is indicated at the termination of the curve.

these tests are shown by the curves marked B in Chart 3. At a level of 2 per cent the extract furnished enough vitamin B for fair growth for the first 40 days, after which there was a decline. Three and five per cent furnished sufficient vitamin B for normal growth.

[1] These percentages are based upon Goldberger's units in which 1 per cent is equivalent to the extract obtained from 18 per cent corn.

The vitamin G content of the extract was determined by feeding it at levels of 2, 3, and 5 per cent in diet 107 thereby allowing the extract to be the only source of vitamin B and G available to the animals. Having already ascertained that as little as three per cent of the extract was an adequate source of vitamin B, any growth in this case would necessarily be due to the vitamin G that it supplied.

The curves marked A in Chart 3 summarize the results obtained. The increase in growth responses of the groups of rats was not proportional to the increase in the percentage of the extract used which would indicate that the extract contained little or no vitamin G. If this is true then rats on diets containing no vitamin G maintain weight at or only slightly below their initial weight for some time and in most cases survive the usual test period of 8 weeks. When the very slight gain in weight of the rats (Chart 2) on diet 107–C30 is compared with these results it is again apparent that the amount of vitamin G supplied by 30 per cent of white corn in the diet is very small.

Tentative Method for the Assay of Foods for Their Vitamin G Content

Following the general vitamin assay methods based on the relationship between the amount of vitamin present and the rate of growth in test animals, Sherman and Sandels (9) reported experiments demonstrating that the growth produced by diets in which vitamin G is the limiting factor, is directly proportional to the amount of vitamin G supplied.

Bourquin, attempting to find a method suitable for vitamin G assay, considered the possibility of using 20 per cent of whole wheat in the diet as an adequate source of vitamin B without supplying significant amounts of vitamin G. She fed graded amounts of autoclaved yeast as a source of vitamin G in addition to a basal diet containing 20 per cent of whole wheat as the only source of vitamins B and G. However, at the usual level of gain of 3 grams per week, the responses were so irregular that conclusions could not be drawn with any degree of certainty. For this reason she discounted the possibility of using whole wheat as the source of vitamin B in an otherwise vitamin G-free basal diet and resorted to the use of alcohol extracts of wheat.

The work reported here was practically completed before Chase (2) and Bourquin (1) made available their results relative to the amounts of vitamins B and G in wheat. Chase found that a daily supplement of 1.0 gram of whole wheat per rat per day as the sole source of vitamin B induced an

average gain in weight of 59.7 grams in 8 weeks. The results in Chart 1 show that 1.18 grams white corn supported an average gain of 59.5 grams in 8 weeks. This would indicate that whole wheat is slightly richer than corn in vitamin B. The work of Mendel and Croll (5) has shown them to have about equal value.

Bourquin (1) found that 0.2 gram of whole wheat per rat six times per week as the sole source of vitamin G resulted in an average gain in weight of 6.6 grams during 8 weeks. In our tests 1.21 grams of corn as the sole source of vitamin G induced an average gain of 7 grams during 8 weeks, showing that white corn contains less vitamin G than wheat.

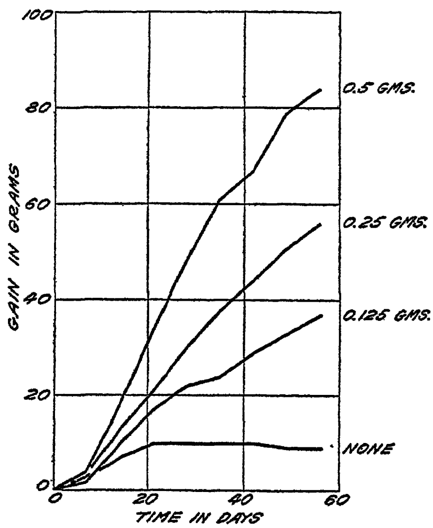

CHART 4. Curves showing average changes in weight of groups of rats fed graded portions of autoclaved yeast as the source of vitamin G. The basal diet used contained only the small amount of vitamin G present in the 30 per cent of white corn used as a source of vitamin B. The amount of autoclaved yeast fed each rat 6 times per week is given in grams at the end of the curve.

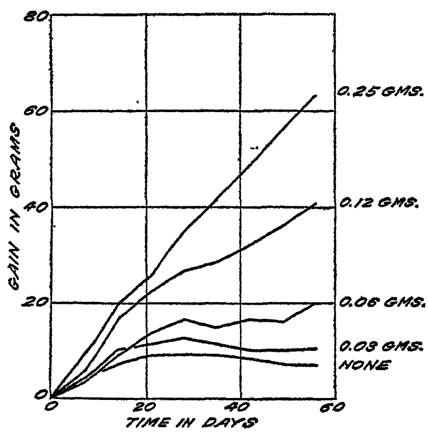

CHART 5. Curves showing average changes in weight of groups of rats fed graded portions of vegetable extract as the source of vitamin G. The basal diet used contained only the small amount of vitamin G present in the 30 per cent of white corn employed as a source of vitamin B. The amount of vegetable extract fed each rat 6 times per week is given in grams at the end of each curve.

Since our results showed that 30 per cent of corn in the vitamin B-free diet of Sherman and Spohn supplied an adequate amount of vitamin B, diet 107–C30 was tested as a possible basal diet to be used in determining the vitamin G content of foods. A number of foods have been tested and the results obtained with autoclaved yeast and a vegetable extract are given here. In Chart 4 are shown the average gain-in-weight curves of

groups of rats fed graded portions of the autoclaved yeast as the sole source of vitamin G. The amounts used were 0.125, 0.25, and 0.5 gram per rat 6 times per week and the corresponding gains in weight were 36.5 grams, 55.5 grams, and 83.8 grams. In Chart 5 are given the results obtained when graded quantities of a well-known vegetable extract supplied the sole source of vitamin B. The amounts fed were 0.03, 0.06, 0.12, and 0.25 gram per rat 6 times per week and the corresponding gains in weight were 10.5, 20.0, 40.5, and 63.5 grams respectively. In the latter case the growth response was very evenly graded to the dose. This product contains a fair amount of vitamin B which may in part account for the uniform results obtained.

These results show that a diet containing 30 per cent of white corn as the sole source of vitamin B may be used in determining the presence and the relative amount of vitamin G present in a foodstuff. It is of course necessary to take account of the fact that small but minimal amounts of vitamin G are present in the corn. This can be done by comparing all results with those obtained with the control animals on diet 107–C30 as a base.

Summary and Conclusions

1. Results of tests with groups of white rats show that 30 per cent of white corn in the Sherman and Spohn vitamin B-free diet does not supply an amount of vitamin G sufficient to promote growth or to prevent the occurrence of symptoms of pellagra.

2. Normal growth results when 30 per cent of white corn is fed as the only source of vitamin B in the Sherman and Spohn diet to which autoclaved yeast has been added to supply vitamin G.

3. An 80 per cent (by weight) alcohol extract of white corn fed at a level of 3 per cent supplies an amount of vitamin B sufficient for normal growth, and contains very little vitamin G. These tests also indicate that rats on a diet free from, or at least containing only minimal amounts of, vitamin G, tend to maintain weight for some time at or near their initial weight. This gives further evidence that the diet carrying 30 per cent of white corn contains very little vitamin G.

4. On a diet in which the vitamin G carried by 30 per cent of white corn was the limiting factor, growth of young rats is shown to be proportional to the vitamin G of the test food fed in addition to this basal diet. Results of tests with autoclaved yeast and with a vegetable extract are given.

5. In cases where conditions do not permit of the preparation or purchase of vitamin B extracts for use in vitamin G assays, white corn to the

amount of 30 per cent of the diet may be used to supply an adequate amount of vitamin B with little addition of vitamin G. When this diet is used all comparisons must be made relative to the growth rate of the controls on the basal diet only.

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THE EFFECT OF PASTEURIZATION UPON THE VITAMIN C CONTENT OF MILK IN THE PRESENCE OF CERTAIN METALS*

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INTRODUCTION

THIS report deals with a study of the effect of certain metals upon the destruction of the antiscorbutic vitamin during the aerobic pasteurization of milk. The data obtained not only are of value in indicating the potentialities of the metals that were studied, but also are probably indications of the percentage of destruction of vitamin C which will occur in the aerobic pasteurization of fresh summer milk.

The problem before the dairy industry today regarding the preservation of the antiscorbutic vitamin in milk may be summed up as one in the combatting of oxidation. Time, temperature, and the repeated contact of air with milk are factors that influence this action. The metals used in dairy equipment also contribute because of the possible catalytic rôle that they may play in oxidation. Metals may also be objectionable if they have appreciable solubility in the milk, or impart a flavor (1). But it is practically axiomatic in the dairy industry that most equipment must be metallic on account of the necessity for good heat conduction as well as for durability. It is obvious, therefore, that an acceptable metal must meet both rigid physiological and mechanical requirements for the maintenance of an unimpeachable position.

Our experiments have been confined to the comparison of copper, tinned copper, and aluminum continuous flow pasteurizers because there is a tendency toward the use of this type of pasteurizer on account of its anaerobic features. For strict comparison of the metals, block tin might have been used instead of tinned copper, but such equipment is practically unknown both on account of the softness of the metal and its high cost.

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Most of the metallic dairy equipment in use at present for processing milk is constructed of tinned copper, except vacuum pans for condensing milk, which are made of naked copper. It may be stated, moreover, that considerable laxity exists in retinning of equipment when worn. Copper is decidedly objectionable in dairy practise owing to the taste it imparts to the milk.

Copper oxide surface is more soluble in milk than bright metallic copper surface (2). Hess and Weinstock (3) have shown that destruction of vitamin C is catalyzed by metallic copper. Keeping the copper bright by constant polishing or use is, therefore, only a partially palliative measure. No direct evidence is available concerning the effect of tin, but presumably from the degree of preservation of vitamin C in some canned vegetables, notably those of an acidic nature such as tomatoes (4), it does not appear that tin itself should be directly under suspicion. Schwartze, Murphy, and Hann (5) have shown that, if hard glass (pyrex) may be taken as a standard, aluminum is also inert and has no deleterious effect upon vitamin C in the boiling of milk. No report of a comparison of copper, tinned copper, and aluminum is available in the literature. The qualities of various metals including tin, copper, and aluminum, from other standpoints of the dairy chemist have, however, been discussed by Hanziker, Cordes, and Nissen (1).

The excellent heat conductivity of aluminum, its low initial and maintenance cost, its ease of fabrication, light weight, and durability[1] are factors that eminently recommend it from an economic viewpoint for use in the dairy industry. In our experience aluminum does not impart a flavor to milk and our analyses indicate that it is not noteworthily corroded by contact with milk. This study of the stability of vitamin C in the pasteurization of milk in aluminum has been proposed by one of us (E. W. S.) to demonstrate the degree of suitability of the use of aluminum in the dairy industry from a public health point of view.

Experimental

The vitamin C destruction in milk samples pasteurized respectively in aluminum, tinned copper, and copper has been determined quantitatively by measuring their values in preventing scurvy in guinea pigs.

The animals subsisted on a scorbutic diet and graded supplementary doses of milk. Six groups of animals were used, as follows:

[1] The pitting of aluminum formerly observed can now be obviated (6) and non-corrosive alkaline cleansers are also available. There is no necessity for the employment of chlorine-containing disinfectants in a modern dairy plant because steam will sterilize if properly used.

a. A negative control group received no supplementary milk.

b. An autopsy control group was fed spinach as a source of vitamin C.

c. A standard comparison group received graded doses of raw milk.

d, e, f. Three groups received graded doses of milk pasteurized respectively in aluminum, tinned copper, and copper.

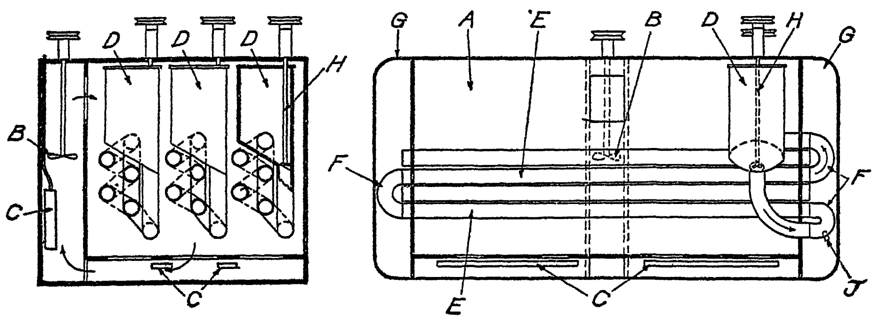
Milk supply. The milk used for this experiment was of certified quality and was obtained from the Braeburn Farms of the Liberty Dairy Products Corporation of Pittsburgh between the middle of May and the middle of September, 1929. At the beginning it was the mixed yield of three cows(one Holstein and two Guernseys). The cows were chosen for a medium, daily quantity of milk. In the later stage of the experiment the milk yield fell below the daily quantity required owing to a long drought and the consequent drying up of the pasturage. It became necessary during this period to add a fourth cow, a Guernsey, with a large daily milk yield. The usual feed of the cows was supplemented twice daily during this period with freshly cut greens or oats, peas and soy beans. The average daily yield of milk was about 18 pounds and varied between 14 and 23 pounds.

The cows were milked about three o'clock in the morning. The milk, after being cooled and bottled to conform to the requirements of a certified dairy, was shipped in ice-packed cases by express to Pittsburgh. It was delivered to the laboratory by truck about 9 a.m. By this method milk was obtained ready for use six hours after the cows were milked. The feeding of the guinea pigs was begun immediately after pasteurization of the milk in the laboratory and all feeding was completed shortly after 6 p.m. The statistical age of the milk as fed, therefore, was about 12 hours.

No facts sufficiently precise are known concerning the comparative qualitative value of pasturage and ensilage in producing milk of high antiscorbutic property. The literature, and our past and present experiment, would tend to indicate that ensilage has been generally superior. The circumstance of a possible depression of vitamin C concentration because of a failure of pasturage was provided for by control experiments conducted simultaneously.

Pasteurizing equipment. The milk was pasteurized in a specially constructed laboratory apparatus (Figure 1). It was designed with a view to obtaining exactly parallel conditions in three pasteurizers each of which was constructed of a different metal. They were contained in a large constant temperature bath (A). The water in the bath was circulated by means of a motor-driven propeller (B). Heat was supplied by electric heaters (C) from the bottom and also from the side close to the propeller of the bath.

Each pasteurizer consisted of a chamber (D) and two long tubes (E) connected together in one closed system and entirely contained within the constant temperature bath with the exception of the connecting bends (F). These bends were outside the bath but were protected from the surrounding atmosphere by removable insulated shields (G). The bends were themselves removable, being held in place by short pieces of rubber tubing. The apparatus was so constructed to facilitate cleaning. Each pasteurizer was equipped also with a motor-driven screw-type propeller (H) of the same



Continuous Flow Pasteurizer

FIGURE 1.

metal as the pasteurizer to keep the milk circulating actively during pasteurization. Care was taken in the use of these propellers to see that the milk was not unnecessarily churned or beaten. A small rubber stoppered hole (J) was provided at the lowest point in each pasteurizer for draining.

Meticulous care was taken in cleaning the pasteurizers to maintain a bright surface throughout at all times. After each pasteurization a hot 5 per cent solution of non-corrosive alkaline cleanser[2] was circulated in the pasteurizer for 15 minutes. After this solution was drained, the tubes and chambers were thoroughly scrubbed with stiff dairy brushes and tap water. Fine steel wool and soap were used to clean all available surfaces but with a sparing use on the tinned copper. As the use of the steel wool gradually removed the tin coating about the circumference of the ends of the tubes of the tinned copper pasteurizer, they were repeatedly retinned with block tin so that a higher standard of maintenance was observed than in ordinary dairy practise on this type of equipment. The extent of corrosion of the aluminum and copper pasteurizer due to the cleaner[2] which contains an inhibitor or anti-catalyst for aluminum corrosion, is shown in Table I.

[2] Oakite Multicleaner.

TABLE I

ANALYSES OF MILK FOR ALUMINUM AND COPPER

	Pasteurization in Aluminum		Pasteurization in Copper	
	Before p.p.m.	After p.p.m.	Before p.p.m.	After p.p.m.
Milk	0.07	0.67	—*	7.2
			—*	8.6
Cleaner solution	0.08	0.41	0.02*	8.2

* No exact quantitative significance is attached to the figures obtained as they averaged less than 0.1 p.p.m. The previous limits of accuracy of the method do not seem to have been proven, and milk runs lowest of all food products reported (0.15 p.p.m. Cu).

Pasteurization of milk. The milk was pasteurized immediately after it was received from the dairy. The milk, at about 8°C., was transferred from the bottles directly into the pasteurizers. Three quarts of milk were used in each pasteurizer. The temperature of the bath had previously been brought to 61 to 62°C. Circulation was started in the pasteurizers immediately. A preliminary pre-heating period of about ten minutes was required to bring the milk to approximately the pasteurizing temperature. This brought the temperature usually to 58.5 to 59.5°C. The pasteurization was timed for 30 minutes from this point. The temperature usually rose to 60°C. or above in the first five minutes of the pasteurizing period and varied from 60.0 to 60.5°C. during the remainder of the process.

It was found that there was a lag of one to two minutes in the temperature rise of the milk in the aluminum pasteurizer. This pasteurizer was, therefore, filled first and emptied last, making the total time of milk in the pasteurizer two to three minutes longer than in the tinned copper and copper pasteurizers. In this way the lag was compensated for and, incidentally, the aluminum was given a slightly more severe test than either of the other metals. The order of filling and emptying the tinned copper and copper pasteurizers was alternated daily so that as between themselves these two metals were given equally severe tests.

When the pasteurizers were emptied, the milk was run directly into ice-packed pyrex glass flasks. It was cooled to below room temperature in these flasks in about five minutes. It was immediately bottled and capped and placed in the refrigerator until used.

The data of the analyses for aluminum of raw milk and of milk pasteurized in aluminum and also those for copper in raw milk and milk pasteurized in copper are shown in Table I.

Selection and maintenance of experimental animals. Young male guinea pigs weighing 200 to 240 grams were purchased from a local breeder. They were placed in individual cages and fed the basal ration (Table II), 35 grams of fresh spinach and 10 to 30 cc. of fresh whole milk daily. This diet differs essentially from that usually employed in scurvy experiments in that it contains no heated skim milk powder. It is equally as efficient in protein as the heated skim milk powder diet and is superior on account of ease of preparation and possibly on account of a better supply of other vitamins(7) This diet supports excellent growth when fed with greens. The body weight and food consumption were watched for 10 to 20 days as well as the condition and type of respiration. Weaklings and those animals showing signs of respiratory disease, which is endemic among commercial guinea pigs, were eliminated. Owing to the failure of complete elimination of undesirable animals within the preliminary period, it was necessary to discard a few animals very early in the experiment proper. Considerable success was had in getting desirable animals, although the percentage of rejections was sometimes high.

The rejected animals were subjected to autopsy for the purpose of checking the quality of the animals purchased. Out of 132 examined, four animals had slight macroscopic lesions of the ribs, presumably scorbutic. The occurrence of traces of scurvy in rejected animals was very probably greater than that in the animals retained. The effect of an occasional animal showing traces of old scurvy, inadvertently introduced into the experment, was minimized by our use of a large number of experimental animals.

Milk was fed by pipette both during the preliminary and experimental periods. This method has an advantage over cup feeding as it insures a constant daily dosage, avoids exposure with a consequent depression of

TABLE II

COMPOSITION OF THE BASAL RATION*

	grams
Ground rolled oats	500
Ground bran	300
Peanut meal	100
Filtered butter fat	49.5
Cod liver oil	0.5
Dried brewer's yeast	20
Salt mixture**	25

* Crude protein, 19.6 per cent. Calcium, 0.565 per cent. Phosphorus, 0.835 per cent.

** Salt mixture, 4.1 grams mono-sodium phosphate, 8.4 grams sodium chloride, 12.5 grams calcium carbonate.

vitamin C content of the milk and prevents the consumption of cream layers. The same type of cages and the same laboratory routine were employed as in former experiments (5). Animals and food were weighed every third day, and fresh trays were supplied.

Attention is directed to examination of the lungs at autopsy. Since our earlier report upon our efforts to obtain experimental animals free from lung complication went to press, and at the conclusion of the experiments herein reported, the work of Tyson and Smith (8) upon chronic lung infection in rats, as well as the work of Coryllos, *et al.;* Henderson *et al.* (9), on dogs appeared. Their work is completely in harmony with our inference from gross pathological examination of lungs of guinea pigs that atelectasis precedes or predisposes to an infection. The effect of insufficient vitamin C in the guinea pig is to restrain it and therefore to increase the effect of the already too potent element of confinement present in any experiment. This factor is very disturbing at times and must be adequately ruled out by application of vigorous methods, or its presence rigorously controlled. Failure to report this condition in the course of experimentation cannot be accepted as evidence of its non-existence in other scurvy work.

Negative controls. The composition of the basal diet is given in Table II. The results of feeding the diet are given at the bottom of Table IV. These experiments indicate that the diet was sufficiently free (possibly entirely free) from vitamin C for use for this purpose. Attention is therefore called to the ease with which a successful scurvy-producing diet may be made from raw materials readily at hand, and without recourse to excessive and questionable devitaminization procedures.

Autopsy controls. The autopsy control animals were fed the basal diet and 15 to 20 gms. of spinach daily. They outgrew the best of the animals receiving supplementary antiscorbutic vitamin only from milk. This speaks for the existence of a considerable range between the apparent protective and the optimum vitamin requirement as well as showing that these animals fed milk only could not be considered as positive controls in the strict sense.

Criteria of assay. The criteria of assay of vitamin C destruction depend upon the production of equal or approximately equal degrees of effect (*i.e.*, scurvy) in respective series of animals from definite doses of milk prepared in different ways. The equality or practical equality was judged by three criteria, namely, clinical course of the scurvy, pathological findings upon post mortem examination, and growth. In biological assay it is necessary to employ a large number of experiments on the same dose to estab-

lish an average. It is also advisable to confirm a conclusion by the use of series averages at other levels of experimentation.

Five stages of clinical scurvy were recognized in addition to the free or doubtful group. It is probably true that many animals diagnosed as free could have been shown by microscopical examination of the tissues to have incipient scurvy. There is no evidence that going through this procedure would have increased the accuracy of the assay. It would have tended to make it unduly involved.

The five clinical stages of scurvy are evaluated, respectively, in the tables by numbers from one to five. The first stage was shown by tender wrists, or tender and swollen wrists. In the second stage, swelling and tenderness of the hind legs appeared, together with the wrist symptoms. In the next stage, "3" in the tables, there was some marked impairment of function of the limbs and the appearance of the scurvy position. In the fourth stage, the hind limbs had become practically functionless, and there sometimes occurred gastro-intestinal symptoms such as prolapse of the rectum or hemorrhage. In the last stage, the animal could hardly feed itself and would fall after a short effort to stand; in other words, it was in or near the moribund state. There was, of course, some deviation in the way in which different animals in the same experimental group reacted as well as the manner in which we were occasionally forced to apply the classification scheme in border-line cases. The five clinical stages of scurvy in addition to a free stage were easily recognized pathologically.

A pathological examination was made on every animal. The jaw and joints (knee) were examined for fragility, the teeth, especially lower incisors, for looseness, and the ribs for beading. The ribs, intestines, joints (knee), and muscles were examined for hemorrhages and oedema. The wrists, scored separately (see second column of tables under "Pathological'), were especially examined for swelling, hyperemia, oedema, signs of old hemorrhage, and overgrowth of bone or old scurvy. Three degrees of lesions were estimated, namely, mild, moderate, and severe, and only rarely intermediate or half stages. In adding up and averaging the numbers indicating the degree of the lesions, we are on insecure ground since there is no proof that fragility of bones, hemorrhage, or oedema should be given equal weight. We have not given, however, high statistical importance to averages, but used them for that which can be seen in a glance without involved computations. For one who has seen considerable scurvy in guinea pigs, it would be equally easy to establish different degrees (say about five) as we did in the clinical examination.

Time of experiments. The time between the beginning of the first and the end of the last experiment was about 5 months. The duration of the majority of the individual experiments was 70 days, and 40 days for a few groups on small amounts of vitamin which gave indications of not surviving as a unit for a longer period. In our experience 90-day tests do not seem to be essential, except possibly for bringing out or magnifying scorbutic states and lesions in animals apparently protected. Forty-day periods are not to be recommended, unless for some specific purpose, if the animals will survive longer as a group.

Results of Feeding Experiments

Raw milk. There are given in Tables III and IV the results of the experiments with the different dosages of raw milk. These experiments have been separated into two groups started at different times. The results indicate that 35 cc. of the raw milk were insufficient to protect guinea pigs from apparent clinical scurvy and that there was about a 20 per cent deterioration in the quality of the milk as the season advanced. The failure of 35 cc. of pasture-produced milk to protect in this instance was not unexpected. The deterioration in the quality of the milk was taken care of as the season advanced by running necessary comparisons simultaneously.

Milk pasteurized in copper. In Table IV are given the data showing the effect of pasteurization of milk in naked copper. Two copper series were run, the second one for confirmation of the striking deterioration of vitamin C as shown in the first. The results compared with those on 14 cc. of raw milk, which animals were killed at the end of 40 days, indicate that much more than 40 per cent of the vitamin C was destroyed. The results of experiments in which 3.5 cc. and 7 cc. of raw milk (diluted to 35 cc.) were fed are so close as to be indistinguishable from the experiments in which 35 cc. of milk pasteurized in copper were fed. We conclude that at least 80 per cent (if not actually 90 per cent) of the antiscorbutic vitamin was destroyed in pasteurizing in the naked copper pasteurizer. It should also be noted that these guinea pigs behaved very much like those receiving no milk. In placing the destruction at only 80 per cent, therefore, we are giving this milk the benefit of all doubt. These experiments confirm Hess and Weinstock (3) and also Flinn (10) as to the marked destructive effect of copper on vitamin C.

No feeding experiments with copper salts were conducted. The course of our experiments was that of typical scurvy and even though dietary copper were capable of producing symptoms and lesions similar to scurvy

TABLE III

THE ANTISCORBUTIC VALUE OF RAW MILK

Dose of raw milk per 300 grams body weight, cc.	Number of animals	Date of Beginning individual experiments	Duration of experiment	Observed Degree of Scurvy		Body Weight		
				Clinical	Pathological	Start	Maximum	End
		1929	Days			grams	grams	grams
35*	4	May 8 to June 10	70	1.4	2.0 and 0.5	294	522	508
35	8	July 14	70	2.75	4.0 and 0.75	288	460	434
28	7	May 8 to June 7	70	2.7	5.3 and 0.6	290	452	437
28	3	July 8	70	3.7	9.3 and 1.7	290	382	372
22.5	14	May 8 to June 10	70	3.1	8.3 and 0.6	298	418	382
17.5	2	June 10	70	3.0	14 and 1.5	288	357	297
17.5	6	July 8	70	3.8	13.8 and 2.5	299	369	339

* Divided into early and late experiments on account of slight deterioration of milk as season advanced.

TABLE IV

THE ANTISCORBUTIC VALUE OF MILK PASTEURIZED IN NAKED COPPER AS COMPARED WITH RAW MILK. NEGATIVE CONTROLS

Dose and Type of Milk per 300 Grams	Number of Animals	Date of Beginning	Duration of Feeding Milk*	Duration of Experiment*	Observed Degree of Scurvy		Body Weight		
					Clinical	Pathological	Start	Max.	End
		1929	Days	Days			grams	grams	grams
35 cc. Copper Pasteurized, 1st series	7	May 20	22	27 to 37 Av. 30	5.0	19.4 and 2.4	298	342	198
35 cc. Copper Pasteurized, 2nd series	16	July 28	19	24 to 35 Av. 28	4.4	18.0 and 2.4	298	324	177
14 cc. Raw milk	11	June 14	40	40†	3.3	10.4 and 1.3	284	354	297
7 cc. Raw Milk Diluted to 35 cc.	14	Aug. 7	19	22 to 36 Av. 28	4.4	19.2 and 2.7	299	319	181
3.5 cc. Raw Milk Diluted to 35 cc.	13	Aug. 4	19	20 to 37 Av. 29	4.3	19.0 and 2.25	294	319	183
Negative Control	17	Aug 5 and Aug. 10	0	17 to 35 Av. 24	3.9	16.3 and 2.4	294	311	171

* Animals fed as long as practicable as a group. Milk was then discontinued and experiments allowed to terminate spontaneously.

† All animals were living and were killed for autopsy at the end of 40 days.

TABLE V

THE ANTISCORBUTIC VALUE OF MILK PASTEURIZED IN ALUMINUM AND IN TINNED COPPER

Dose and type of milk per 300 grams body weight	Number of animals	Date of beginning	Duration of experiments	Observed Degree of Scurvy		Body Weight		
				Clinical	Pathological	Start	Maximum	End
		1929	Days			grams	grams	grams
45 cc. Aluminum pasteurized	15	June 27 to July 3	70	2.5	4.6 and 1	291	502	486
45 cc. Tinned copper pasteurized	12	June 27 to July 3	70	3.6	7 and 1	288	435	415
35 cc. Aluminum pasteurized	7	May 20	70	2.3	5 and 1	302	450	438
35 cc. Tinned copper pasteurized	9	May 20	70	2.9	8.3 and 1.6	303	372	358

(which it is not known to do) the elucidation of such a mechanism of its action is aside from the present purpose, and would, if it were true, not detract from the full significance of these data.

Milk pasteurized in aluminum and in tinned copper. In Table V are given the results of feeding milk pasteurized in aluminum and tinned copper. The autopsies on these animals were performed on a number at one time without knowledge of identity of the respective experiment. The animals were placed on the respective experiments without selection or any preference. The results when compared with those in Table III show between 20 and 40 per cent destruction of vitamin C. The results with the pasteurization in aluminum are more favorable than those with tinned copper both as regards the amount of clinical and pathological scurvy and the body weights. This conclusion was also reached with ease by watching daily the course of these experiments. The exact amount in favor of the aluminum could not be stated mathematically from series of this type without further experimentation. This observable difference is consistent with the findings on the naked copper experiment, inasmuch as evidence of slight wear was observed on certain parts of the pasteurizer inaccessible to tinning. These experiments also bear out our previous conclusion that there was some deterioration in the quality of the milk as the season advanced.

Conclusions

The effect upon the vitamin C content of pasteurizing fresh milk aerobically in aluminum, in tinned copper, and in copper tubular pasteurizers has been studied. The destruction of vitamin C was placed at 20 to 40 per cent in aluminum. This is greater than the destruction previously obtained upon boiling milk for 5 minutes and is rather to be expected as the duration of exposure in pasteurizing was much longer and strictly aerobic. No pasteurizing experiments were run in hard glass (pyrex) because equality of glass and aluminum had been previously established in the boiled milk experiments.

The effect of pasteurizing milk in tinned copper was slightly greater than that found with the aluminum. The difference is less than the order of the increments of dosage employed. The effect was noticeable both during the progress of the experiment and upon post-mortem examination of the animals. Each of the two series of experiments upon milk pasteurized in copper was run respectively and simultaneously with two series of experiments upon milk pasteurized in aluminum, so that possible deterioration of raw milk as the season advanced does not enter. This effect we believe

TABLE V

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Dose and type of milk per 300 grams body weight	Number of animals	Date of beginning	Duration of experiments	Observed Degree of Scurvy		Body Weight		
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is referable (*vide infra*) to the small amount of copper exposed to the milk when in the tinned copper pasteurizer.

The pasteurizing of milk in naked copper resulted in a destruction of at least 80 to 90 per cent of the antiscorbutic vitamin, but since the exposure to copper as well as the aerobic pasteurizing process both tend to destroy the vitamin, all of the 80 to 90 per cent cannot be charged to copper. Since both acted simultaneously, attention is called to the fact that less than the stated 20 to 40 per cent must be charged to the aerobic pasteurization process itself because copper has the greater effect.

The practical importance of these experiments is that copper is again shown to be unsuited for construction of dairy equipment so far as the conservation of vitamin C is concerned. Tinned copper is likewise unsatisfactory both on account of the impracticability of replating of worn inaccessible parts of equipment and of the laxity in retinning by the dairy trade. Glass (pyrex) is assumed to represent an inert material and by previous experimentation with boiled milk no difference was found between it and aluminum. Aluminum is therefore as satisfactory a material as we have for the construction of dairy equipment as regards the destruction of vitamin C. We have reason to suspect that block tin would not be satisfactory.

From the standpoint of corrosion the position of aluminum as regards the extent of its entrance into the milk was satisfactory; 0.6 parts per million; normally present 0.074 parts per million. The objectionableness of copper is no longer a moot question even from the standpoint of the dairy chemists themselves, although small amounts—0.25 to 0.50 parts per million—may be normally present in milk. The amount of tin which was actually present is not known. Some undoubtedly wears off in cleaning. Tin and aluminum are both pharmacologically superior to copper for use as food containers. It is hard to conceive that hard glass (pyrex) would contribute substances (aluminum, etc.), to milk although the methods of analysis are not yet capable of answering this point. For the present, therefore, if one were to insist upon universal freedom of food containers and manufacturing equipment from corrosion, it must be sought in materials not now practical or available.

The experiments herein reported are not inconsistent with the theory that anaerobic pasteurization would reduce the vitamin destruction, possibly to zero, even in the case of copper. It should be pointed out in this connection that even though the vitamin destruction were accordingly reduced we would for a sound public health policy still be interested in securing construction material with the greatest negative potentialities.

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FURTHER OBSERVATION OF THE EFFECT OF LIGHT ON THE SYNTHESIS OF VITAMINS

By

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IN A previous article one of us (1) stated that vitamin A was present in greater amounts in wheat seedlings which had been exposed during the growing period to the open sunlight than in those grown in darkness; that the ultra violet, carbon arc, or the Mazda lamp, all caused an increase in the amount present as compared to the etiolated sprouts, but an amount less than that found in the sunlight-grown plants. The etiolated sprouts contained very little of the vitamin, if any. These findings compare favorably with those of other investigations mentioned in our previous article. Since that time Moore (2), after a carefully planned piece of work, states that seeds germinated and fed in complete darkness contain measurable, though small, amounts of this substance.

Vitamin B (B and G) was reported at that time as not increasing its amount during the germination period. While tests have not been carried out since the recognized division of B into two parts, in all probability had either increased it would have been noted in the previous tests. Vitamin C, we felt, increased somewhat more in seeds germinated in the light than in those kept in darkness. Since that time Matsuoka (3) has reported similar findings. However, Honeywell and Steenbock (4) and Eggleton and Harris (5) believe that germination alone is responsible for this result.

Chick and Roscoe (6) state that spinach contains little vitamin D. Boas (7) reports none present in winter-grown spinach. Stepp (8) states that germination produces no vitamin D, while Voltz and Kirsch (9) and Schittenhelm and Eisler (10) state that seedlings grown in the dark or in the sunlight possess antirachitic properties. While these reports are seemingly contradictory, they are somewhat parallel with our results reported in this article.

A number of interesting articles somewhat related to this problem have been reported. Dye, Medlock and Christ (11) state that the outer green leaves of lettuce are far superior to the colorless inner leaves in their vitamin A content. Steenbock and Sell (12) have stated the same for cabbage.

Christ and Dye (13) find similar results for green and uncolored asparagus shoots.

All these reports seem to indicate that vitamin A is in some way associated with the chlorophyll of the plant. However, other recent reports seem to contradict this assumption. House, Nelson, and Haber (14) and Morgan and Smith (15) find that green tomatoes contain less vitamin A than colored ones, regardless of whether they were ripened in the sunlight, in darkness, or artificially in an ethylene atmosphere. The latter authors state the same for red bell peppers.

Coward (16) reports that when leaves lose their green color and become yellow their vitamin content increases.

We have demonstrated (unpublished data) that yellow beans are as potent as related green varieties. It has often been suggested that the vitamin was associated with carotin, yet others have found potent sources quite devoid of this substance. These contradictions tend to prove that there is yet much to be learned in regard to the chemical composition of the vitamin. It is safe to postulate that the vitamin carrier is synthesized under conditions similar to the production of chlorophyll, but when the latter is transformed during the period of ripening, the vitamin retains its form. Considerable data in connection with this subject have accumulated since our last paper, and at this time we desire to report on certain aspects of the problem. The phases to be considered are: 1.—the effect of colored lights on the synthesis of vitamin A; 2.—the effect of blanching tissue which has previously become potent; 3.—the possible migration of the vitamin from one portion of the plant to another; and 4.—the effect of light upon the synthesis of vitamin D.

Experimental

The first series of tests are all concerned with vitamin A. A single brief description of the technique employed will serve for all the tests. The ration consisted of dextrin, 70.5 per cent; alcohol extracted and heated casein, 18 per cent; Fleischmann's dried yeast, 8 per cent; McCollum's No. 185 salt mixture, 3.5 per cent. The entire mixture was irradiated in thin layers prior to its use. The test animals were young rats from our own colonies weighing about 50 grams. The cages, which were of the usual type, contained four or more rats each, the animals having been selected to be comparable as to age, sex, and litter origin. In all cases the basic ration was fed until the growth curve of the animals became flat, at which time the supplements were fed directly to the animals in such a manner as to keep the food consumption of the various lots comparable.

The Effect of Blanching of Vegetables

Our previous tests having demonstrated that seedlings, etiolated by being kept continuously in the dark, were much less potent than seedlings raised in the sunlight, the next question suggested was whether a vegetable raised in the sunlight, then whitened by exclusion of sunlight, likewise loses its vitamin potencies. Wheat seedlings were raised in a washed sand medium until the green plants were about two inches in height. One tray was then placed in a dark room until practically etiolated, while the other tray remained in the sunlight. A third tray was kept through the entire time in darkness. Three cages of rats depleted of vitamin as previously explained were fed the seedlings. Lot 1 received 10 green sprouts per rat per day, lot 2 received an equal number of the seeds etiolated after growth, and lot 3 received a like number of seedlings germinated in complete darkness. The average of several such trials is shown in the following table. The first series was carried out in early winter and the second in early summer.

Table I

Type of Supplement	Days Fed	Average gain in weight
Sunlight-grown	35	25
Blanched	35	19
Etiolated	35	9
Sunlight-grown	70	51
Blanched	70	42
Etiolated	70	16
Green leaves of celery	55	76
White leaves of celery	55	67

These results indicate that once the vitamin has been synthesized it is not destroyed with the disappearance of the chlorophyll.

Such a question is of interest in the use of celery which has been etiolated after attaining full growth. A test of green and white outer leaves of celery was made in the same manner. The results are shown in Table I.

This is another indication that after once acquiring potency these leaves retain their vitamin. If some allowance be made for the increase of vitamin in the green seedlings which received the longer exposure, the results of the two seem to be practically equal.

Place of Formation or Storage of the Vitamin

An attempt has been made to determine whether the vitamin is equally distributed throughout the tissue of the germinating seeds. Wheat seed-

lings were grown in the sunlight in sand medium as before. When the sprouts were about two inches above the level of the sand they were used in the following tests. Rats depleted of vitamin A were divided into three cages. Lot 1 received 10 sprouts per rat per day, lot 2 received only the green tops of 10 sprouts per rat per day, and lot 3 received the seed remnant from which the tops had been removed. Table II records results of these tests.

Table II

Type of Supplement	Days Fed	Average gain in weight
Whole green sprouts	35	25
Tops	35	29
Seed remnants	35	9
Whole green sprouts	40	50
Tops	40	40
Seed remnants	40	10

The two groups of tests again represent the averages of lots, the first of which were carried out in cold weather and the second in early summer. These results indicate that the vitamin is formed and stored largely in the portion of the plant exposed to light and it evidently indicates that the vitamin does not migrate to the other portions of the plant. This would explain also why the inner white leaves of lettuce, or cabbage, are not so potent as the green outer leaves.

To Determine the Effect of Colored Lights or Light Waves of Different Length Upon the Synthesis of Vitamins in Growing Plant Tissue

In order to determine the comparative efficiency of long and short wave lengths upon the synthesis of vitamins, special glass filters were secured from the Corning glass works. The first was a blue glass (green glass G403ED) which transmitted waves of 435–490 μm, theoretically removing the greater portion of the long-wave red spectrum. The second was an infra red glass (infra red G554FF) transmitting only waves of 626–720 μm, removing the greater portion of the more visible spectrum. Wheat seedlings in sand media were grown under these filter screens in the open sunlight until the sprouts were approximately two weeks of age. Using the same technique as before, ten of the seedlings raised under the blue glass were fed to each of the rats in one cage each day. Ten seedlings per rat per day from under the infra red glass were fed to the second cage of rats

each day. The third cage received 10 seedlings per rat per day, raised in the open sunlight and were used as a control group. This experiment has been repeated ten times at various seasons of the year, and for various lengths of time. While the results are not always entirely comparable, probably due to the season of the year and the consequent variation in illumination, the general trend of results is the same. An average of the entire series representing over 40 rats in each case gives the following results.

Type of Supplement	Days Fed	Average gain in weight
Sunlight	46	53.
Green Glass	46	43.5
Red Glass	46	38.

An inspection of these data would indicate that the short wave was more important in this synthesis. Certain other factors need to be considered before drawing these conclusions too definitely. First, the seed bed and growing conditions remained more constant under the glass filters than in the open air, which should favor their potency. Second, the intensity of illumination must be altered. Either filter must reduce this effect. According to the manufacturer's investigation, the visible intensity is entirely removed under the infra red, yet we find the seedlings from this group only slightly less potent. Third, the short waves cannot be considered as entirely responsible for the changes, for again the infra red filter is supposed to screen out largely this end of the spectrum.

Evidently there is a series of factors involved, probably interrelated or affecting the others, namely, intensity of light, type of light wave, temperature, and possibly others. There is much to indicate that light waves beyond the spectrum are effective.

Effect of Light Upon Synthesis of Vitamin D

To test the effect of germination in light upon the formation of vitamin D, seeds were germinated as before, one lot in open sunlight, one under green glass, one under red glass, and the last in darkness. Five cages of rats, selected for the vitamin D work, were kept in a semi-darkened room and fed the Steenbock and Black (17) ration. When a rachitic condition was established by technique formerly outlined by us (18), the supplements were added. Cage 1 received 8 sunlight germinated seedlings per rat per day, cage 2 received an equal number of those grown under green glass, cage 3 received an equal number of those grown under red glass, cage 4 received an equal number of those grown in darkness, and the control lot

in cage 5 received only ungerminated seeds. Twelve days thereafter the skiagrams of all groups showed no cures. A second series of skiagrams taken 18 days after adding supplements showed those that had received seedlings from sunlight practically cured. No other series was completely cured, and after 24 days those receiving seedlings from under green glass were only partially cured, those under red glass showed no improvement, and those receiving etiolated seedlings and ungerminated seed showed a very critical condition. An examination of the data indicates that green leaves are not good sources of vitamin D, that seedlings grown in the open sunlight do synthesize vitamin D to a certain extent, and that no appreciable increase is noted in the absence of brilliant sunlight. Possibly we are dealing here with a mild irradiation rather than with synthesis, though an irradiation of dry seeds produced no parallel results. A parallel series of results with asparagus shoots, using as high as eight grams per rat per day, failed to produce a curative action in any case.

Another series of tests designed to compare the potencies of yellow and green beans, yellow carrots, and red beets failed to show any preferences, in fact, even when these substances made up fifty per cent of the ration, a cure was not effected in fifteen days. These tests tend to prove that vegetables cannot be depended upon as a source of the antirachitic factor.

Conclusions

1.—The results seem to indicate that after green leaves have become a potent source of vitamin through exposure to the sunshine, potency is not destroyed with the destruction of the color in blanching.

2.—As the vitamin forms, it seems to be stored in the portion of the plant most exposed to the sunlight and it is not transferred to the other portions of the plant, and the most actively growing portion, if etiolated, may be devoid of potency.

3.—It is evident that a number of interrelated factors are active in the synthesis in light. Neither wave length nor heat can be considered solely responsible.

4.—Wheat seedlings germinated in open sunlight possess some antirachitic properties; grown under green glass there is slight potency, while no beneficial results were obtained from seedlings grown under red glass or in darkness.

5.—Tests of green and yellow beans, carrots, and beets, failed to show any relationship between potency and color.

6.—Vegetables are a very poor source of vitamin D.

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THE MINERAL EXCHANGES OF MAN

I. ORGANIZATION OF METABOLISM WARD AND ANALYTICAL METHODS

By

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INTRODUCTION

UP TO the present time studies of the mineral exchanges of man, in the majority of instances, have been concerned with only two or three of the ash constituents. Many years ago Bunge (6) stated that the ingestion of an excessive amount of potassium caused a loss of sodium from the body. If this be true, then an interpretation of the behavior of any single mineral substance demands a knowledge of the simultaneous behavior of others, particularly of those which are known to be physiological antagonists, or of those which possess the power of displacing other ions from the body.

In the past two and one-half years we have made an effort to carry on more comprehensive observations of the mineral exchanges of man, observing the simultaneous behavior of sodium, potassium, calcium, magnesium, iron, nitrogen, and phosphorus, in the hope of discovering some of the fundamental relationships between these substances. The subjects studied have included normal men, patients with pernicious anemia before and during the blood regeneration induced by liver therapy, a patient with erythremia during blood destruction resulting from the administration of phenylhydrazine hydrochloride, certain patients with osteoporosis and degenerative arthritis, a patient with chronic jaundice of five years duration due to biliary cirrhosis, and an oedematous patient with chronic hemorrhagic nephritis during diuresis induced by the administration of potassium citrate. Further studies are in progress.

In order to carry out a program of study of the mineral exchanges of human subjects it is necessary to have a metabolism ward and diet kitchen especially adapted to this work. From the beginning, the Medical Clinic of the University of Rochester has possessed such a special metabolism unit in the Strong Memorial Hospital. One of us, W. S. M., having been

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trained by E. F. DuBois in the methods of the metabolism ward in Bellevue Hospital, copied the procedures used there as completely as possible. These have been described in a paper by Gephart and DuBois (8).

The metabolism unit consists of a well equipped kitchen flanked on each side by two rooms, each containing two beds. Between these rooms and the kitchen there are no doors, but glass partitions permit the nurses, as they work in the kitchen, to keep patients continually under observation. Each room has a small closet containing a commode chair in which is a weighed container for reception of excreta, for the use of patients who can get out of bed. For bed-ridden patients, a weighed bed pan is used. In the utility room adjoining, there are no facilities for the disposal of excreta so that it is impossible for specimen containers to be emptied by careless orderlies or cleaning women. Orderlies are forbidden to enter the unit except in company of a nurse. The personnel of the unit consists of a head nurse, a pupil nurse, a pupil dietition, a night nurse, and two chemical technicians. An assistant resident physician is assigned to this unit in addition to his other routine duties. The methods of weighing patients on a "silk" scale, of collecting urine specimens in separate bottles, of collecting feces, separating periods by carmine ingestion, weighing, and preparation of foods, are, in the main, identical with those described by Gephart and DuBois (8). The differences and exceptions will be noted in detail. Many helpful suggestions have been obtained from the paper by Bauer and Aub (5).

Collection and Preparation of Excreta

Each voiding of urine is kept separate in a tightly stoppered 20 oz. bottle, with a little toluene. The date, hour and minute of voiding are recorded in pencil on the etched area on the side of the bottle. It is stored in a refrigerator until ready to be sent to the laboratory. As the nurse receives each specimen, she records the fact on an invoice which is to be sent to the laboratory, noting the approximate volume of urine obtained by measuring the height of the urine on a scale. When the 24-hour urine is subsequently mixed and measured in the laboratory the loss of a specimen may be detected by the invoice and, at least, the approximate amount lost, is known. Mineral balances in this study have never been calculated on periods of less than five days. After measuring the mixed 24-hour specimen an aliquot part is saved and subsequently combined with the other daily aliquots corresponding to each period. Another portion is used for daily determinations of nitrogen and for such routine clinical examinations as are desired.

The moist weight, to the nearest gram, of each freshly passed stool is quickly obtained by weighing it directly in its container, the tare of which is known. It is then mixed to a paste with alcohol, and dried in the container on a steam bath. The dry weight of the stool is obtained by weighing it in its tared container before scraping it out. The portion of dry stool which adheres is small but is included in the calculation made from subsequent analysis of the portion removed. Weighings are made to 0.1 gram. The head nurse has the responsibility for separating stained from unstained fecal matter at the beginning of each period. In order to make this separation accurately the stool should be formed, hence cathartics should not be used. During the period bowel movements are secured if necessary by simple water enemas. Mineral oil is never used because it interferes with the preparation of the dried stool for analysis. The mixed specimens of dried stool are ground to a meal-like consistency with a porcelain pestle in a Coors mortar. The whole is saved in a tightly-stoppered container. From this an aliquot part of the whole for the period is removed and ground till it will pass a 20 mesh sieve, from which powder samples may be weighed for either dry or wet ashing. Occasionally a stool is encountered which contains a considerable amount of unabsorbed fat, so that it cannot be so finely ground. In this case, large samples are ashed to compensate for the slightly lesser accuracy of sampling.

The error involved in the changing water content of the dried stools, under the conditions of storage, must be considered. The maximum variation observed in one year amounted to about one per cent.

Some of the patients with pernicious anemia were quite ill in the early stages of the study. Such patients occasionally refused a portion of the food. It was at times impossible to weigh back rejected food in such a manner as to make an accurate subtraction of its mineral content from the calculated intake for the day. In such cases the rejected food was ground finely and added to the stools, without subtraction from the calculated intake. Vomitus was likewise added to stools, in the few cases in which emesis occurred.

The water balance of the body is intimately connected with its mineral exchanges. In nearly all cases a record of water balance was kept as follows: the *intake* was taken as the sum of all liquids plus an amount of water equal to the weight of the food *as served;* the *output* was the sum of all urine voided plus an amount of water equal to the weight of freshly passed stools, vomitus, and sputum, plus the water lost through the skin and lungs. An estimate of the latter item is obtained by correcting the differ-

ence between the other items of intake and output for the net change in weight during 24 hours. For example, a patient weighed 90.00 kg. at the start and 90.25 kg. at the end of a day, a net gain of 250 grams, ± 10 gm. The total intake was 2580 cc. The total output through kidneys and gastro-intestinal tract was 1260 cc. The difference, 1320 cc., corrected by subtraction of 250 gm. water added to the body gives 1070 cc. as an approximation of the water lost through the skin and lungs. The errors in estimating water balance in this way are not large. Preformed water constitutes a large percentage of the ingesta. Of the solids of the ingesta, the indigestible residues excreted, will, in the long run exactly balance those ingested, and will correspond closely day by day when the diet is fairly uniform, or, if retained or lost in excess, will appear in the net change in weight of the subject. Of the digestible solids, foodstuffs of the ingesta, the portion unabsorbed will affect the balance precisely as the indigestible residues; the portion absorbed will either be deposited as such and corrected for by the change in weight or, if oxidized, will give rise to very nearly the same weight of water of combustion (16). Unless the complete energy balance be known it is impossible to calculate water of combustion more accurately, since the amounts of carbohydrate and fat burned cannot be determined. The calculation of the water balance is a most valuable check upon the accuracy of the nursing and dietetic procedures. It provides a means of doubly checking all operations, and of detecting errors.

In the preparation of foods the utmost care was taken to prevent losses. In general, the precautions described by Bauer and Aub (5) were taken. Foods cooked in water were prepared in individual servings, the water reduced to a minimum and special pains taken to secure ingestion of all the water which remained after cooking. The list of *foods* used in the study of mineral exchanges was carefully selected. The various items were chosen for relative constancy of composition and the availability of reliable analyses of mineral content. In some of the earlier experiments the ash constituents of each day's food were calculated from tables. A comprehensive experiment was carried out, which had the double purpose, first, of determining the accuracy of such calculations, and second, of working out menus of ten diets the mineral constituents of which were actually determined by analysis of the entire food for a day, ground, mixed, dried, sampled, ashed, and analyzed by exactly the same methods as were applied to the excreta (see below). The data obtained will be presented below. It may be said at this point that in the case of iron, magnesium, potassium, phosphorus, and nitrogen, the results agreed very closely with those

calculated from the tables of Sherman (17). In the case of calcium, the amounts found on analysis were consistently below the amounts calcu-

TABLE I

Diets	1 gms.	2 gms.	3 gms.	4 gms.	5 gms.	6 gms.	7 gms.	8 gms.	9 gms.	10 gms.
Apple		150		150	50					
Banana			30			40	150	100	40	100
Beef Rd.			30			100			40	
Beef tend'l'n.					100				120	
Bread	80	70	40	80	60	60	60	55	60	50
Butter	25	45	30	25	40	35	20	25	30	20
Cabbage	80									
Carrots		80		80						
Cauliflower					70					
Celery	20		20							20
Cheese	30	20		30		30	30	30		40
Cocoa								3		
Cod steak		90								
Cream, 4X	110		60	80	80	120	135	90	100	80
Egg	125	180	160	80	170	80	110	100	80	80
Farina			15			15		15		
Flour		5						8	3	
Grapefruit	100				100					
Grape juice		100								
Haddock							80			
Lamb chop	100									125
Lettuce	20	20	20	20	20	20	20	20	20	
Milk	120	200	190	280	230	300	170	430	340	260
Oatmeal	15			15					15	15
Onion		70	90			30			90	
Orange		80	200	80	60	150		100	150	100
Parsnip						70		70		
Pear				80						
Peas					80		80			
Pineapple							50			
Pork chop			120							
Potato	70	70	70	70	70	70	70	70	70	70
Prunes										20
Rice			10			20	20		10	
Shredded Wheat		20			20		20			
Spinach	80			70						70
Squash			70			70				
Strawberries	100				100					
Sugar	30	35	45	40	25	25	35	35	25	30
Tapioca		5					5			
Tomato	80	100	60	60	50	50	160	100	60	
Veal steak				120				90		

lated from the tables, and in the case of sodium, the amounts found were consistently above the amounts calculated; the calcium being 15.5 per cent below Sherman's figures, and the sodium 18.6 per cent above. The data given by Bauer and Aub (5) confirm our findings in the case of calcium. In Table I of their paper, their analyses of 18 items are compared with those of Sherman, the results of the analyses at the Massachusetts General Hospital being 12.8 per cent below those of Sherman, the average of a comparison of the whole 18 items.

Table I gives the composition of the ten diets used, expressed in terms of raw weights. It will be noted that a considerable variety of foods was used. Some of these are undoubtedly of variable composition, but the most variable items contribute a small percentage of the total ash constituents. Furthermore, it is apparent from the results in Table II, that, with

TABLE II

Diet No.	Cal.	P gm.	F gm.	C gm.	N gm.	P gm.	Na gm.	K gm.	Ca gm.	Mg gm.	Fe gm.
1	1984	64.2	126	134	10.3	1.076	1.565	2.620	0.781	0.257	0.0156
2	1959	60.9	96	199	9.7	1.294	1.585	2.427	0.664	0.203	0.0112
3	2003	67.4	116	158	10.8	1.070	1.003	2.483	0.471	0.178	0.0093
4	1998	66.4	108	176	10.6	1.223	1.728	2.670	0.839	0.247	0.0136
5	1976	64.0	117	155	10.3	1.140	1.213	2.253	0.566	0.200	0.0158
6	1979	65.0	110	168	10.5	1.254	1.350	2.590	0.809	0.198	0.0109
7	1996	65.4	104	185	10.5	1.194	1.416	2.580	0.701	0.249	0.0092
8	1978	64.6	114	159	10.3	1.224	1.342	2.540	0.936	0.200	0.0120
9	2011	68.9	121	147	11.0	1.069	1.082	2.440	0.612	0.206	0.0124
10	1986	63.5	121	146	10.2	1.195	1.305	2.760	0.848	0.202	0.0149
Total found					104.2	11.739	13.589	25.373	7.227	2.140	0.1249
Total calculated					111.2	11.939	11.464	26.365	8.557	2.155	0.1252
Per cent difference					−6.3	−1.7	+18.6	−3.8	−15.5	−0.7	−0.24
Per cent agreement of duplicate diets					±0.4	±0.1	±0.7	±1.7	±1.6	±1.0	±2.4

such a variety and with combinations of 5 or more diets in a period, the errors tend to balance each other.

Table II gives the composition of the ten diets compared with the mineral content as found by analysis. By comparison of the totals found and calculated for all ten diets one may estimate how much of a correction must be applied when a calculation is made from Sherman's tables (17) especially for calcium and sodium. It is quite possible that the geographical area from which the food supplies come will determine the magnitude of this difference. In view of this possibility it would be wise for workers in other regions to make similar tests. It is interesting to note in the same table the close percentage agreement between the analyses of two separate preparations of each diet, made in different years and at different seasons. The greatest variation was in the case of iron ± 2.4 per cent. In the later studies of mineral exchanges the intake for each period was calculated from the data in Table II. With ten diets analyzed it was possible to give a different diet on each day without having it fall monotonously on the same day of the week. All diets were calculated to 2000 calories. If it was desired to vary the total calories, this was done by an increment of some simple fraction, and each item on the menu was weighed out in the same proportion. That is if the menu for 2000 calories called for 100 grams of some item, for 2500 calories, 125 grams of this item would be weighed out, and a corresponding correction of the total ash constituents of the day's food would be made.

Analytical Methods

The methods of analysis employed were the following: nitrogen was determined by the Kjeldahl method; phosphorus by the method of Epperson (7); sodium by the method of Barber and Kolthoff (3, 4); potassium by the method of Shohl and Bennett (20); magnesium by the method of Epperson (7); calcium by the gasometric method of VanSlyke and Sendroy (22); and iron by the electrometric titration of King and Washburne (12). The complex nature of the material analyzed made it necessary to modify slightly some of the methods in order to obtain accurate results. Modifications, precautions as to technique, methods of ashing, etc., are cited below.

Ashing. Both dry and wet methods of ashing were employed in the preparation of samples for analysis. Of the two methods dry ashing gave the more uniformly reliable results. Large enough samples (20–30 gm.) could be ashed by this procedure to make errors due to sampling unlikely, and to provide enough material for all analyses. Moreover, the ash ob-

tained was more nearly completely soluble in dilute hydrochloric acid than that obtained by the use of the wet method. This greatly facilitated the preparation of the solutions from which the analyses were made. All diet samples were dry ashed, while many samples of urine and feces were ashed by the wet method. Both procedures are described.

Wet ashing was carried out in 800 cc. Kjeldahl flasks with a digestion mixture consisting of sulfuric and perchloric acids. Fifteen cubic centimeters of concentrated sulfuric acid (sp. gr. 1.8) and approximately the same amount of 60 per cent perchloric acid usually sufficed to ash 4 to 6 gms. of the dried material (feces or diet). The perchloric acid was added in quantities of 4 or 5 cc. at intervals during the digestion and the ashing considered complete when the sample had been reduced to a nearly colorless melt in the bottom of the flask. Considerable foaming occurred during the early stages of the digestion and careful regulation of the heat was required to prevent loss of material from the flask. In the case of urine, 1 liter was evaporated to a small volume in a large pyrex beaker on a steam bath, and quantitatively transferred to a Kjeldahl flask. Twenty to 30 cc. of concentrated sulfuric acid were added and the mixture heated cautiously over a free flame until fumes of sulfur trioxide were evolved. Perchloric acid was now added and digestion continued until ashing was complete. The ash and remaining acids were dissolved as completely as possible in about 150 cc. of dilute hydrochloric acid (1 to 10), filtered and the filtrate diluted with water to a volume of 200 cc. in a volumetric flask. When calcium was present in considerable amounts, calcium sulfate remained as a difficultly soluble residue. This together with any other residue remaining on the filter was fused with sodium carbonate and a separate solution of the fusion mixture prepared.

Dry ashing of stool and diet samples was carried out in 250 cc. porcelain crucibles placed in a large electric muffle furnace, the temperature of which was increased slowly to avoid active ignition and thereby decrease the possibilities of loss by spattering or convection by currents of gases. Volatilization of sodium and potassium salts was guarded against by keeping the maximum temperature of the furnace between 500 and 600° C. Ashing at this temperature is slow. At the end of 12 to 14 hours, the ash was digested with dilute hydrochloric acid (1–3) and any residue remaining removed by filtration through Munktells No. 1F ashless filter paper. The washed residue if it contained carbon was placed, together with the filter, in a porcelain crucible and treated with a few drops of nitric acid or superoxol (30 per cent hydrogen peroxide) and reashed. The extraction was re-

peated as before and the final residue which remained (usually very small in amount) after burning the filter was fused with sodium carbonate. The solution of the fusion mixture (as in the case of that prepared from wet ash) was stored separately from the main ash solution and used in connection with the latter only in determination of calcium, magnesium, and phosphorus.

Dry ashing of urine was accomplished as follows: 2 liters of urine in a large pyrex beaker were evaporated on a steam bath to a volume of 200 to 300 cc. treated with 50 cc. of concentrated nitric acid, the beaker covered, and the mixture allowed to digest for six to eight hours. The residue was now quantitatively transferred to a large porcelain casserole and as much water and nitric acid as possible removed by prolonged heating on the bath. Ashing was then begun by cautiously heating the casserole on a hot plate until most of the nitro-compounds had been decomposed and the residue carbonized. The casserole was finally transferred to the furnace, ashing completed and solutions prepared as described for stool and diet samples.

Nitrogen. Urine nitrogen was determined by the Folin-Wright simplified macro-Kjeldahl method (9). Nitrogen in diets and feces was determined in 1 to 2 gm. samples of the dry pulverized material by an official method (1).

Phosphorus and magnesium. The method of Epperson proved satisfactory for the determination of phosphorus and magnesium. After removal of calcium by precipitation with an excess of ammonium oxalate from a faintly acid solution (pH 4.8 to 5.2) (19) the filtrate was evaporated to dryness on a water bath and treated with an excess of concentrated nitric acid (10 to 15 cc.) to decompose ammonium salts (15). The nitric acid was removed and salts dehydrated as completely as possible by evaporation to dryness on the water bath. A small amount of insoluble silicic acid was usually formed at this point. The residue was taken up in a little dilute hydrochloric acid and any silica present removed by filtration through an asbestos Gooch. Five cc. of a 5 per cent solution of citric acid (15) were added with the magnesia mixture or phosphate solution to prevent the precipitation of iron and aluminium. A single precipitation of the magnesium ammonium phosphate gave sufficiently accurate results for the purpose of this study. Duplicate analyses agreed within 1 per cent. The use of filter paper was obviated in collection of the precipitate by filtering through weighed porcelain filter crucibles with fritted porcelain

bottoms.[1] Ignition of the crucibles for 1 hour in an electric furnace at about 800° C. was found sufficient to convert the magnesium ammonium phosphate into magnesium pyrophosphate.

Sodium. Phosphorus, and in the case of some of the stools which were wet ashed, potassium, interfered with the determination of sodium (3, 4). Phosphorus was readily removed by magnesia mixture[2] from samples of diet and urine ash. The small amount of sodium present in stool ash necessitated the use of relatively large samples for each determination. The high concentration of calcium in such samples resulted in the precipitation of most of the phosphorus as calcium phosphate. The amorphous character of this precipitate made its removal and washing difficult by the ordinary process of filtration. It was therefore removed by centrifuging in 100 cc. centrifuge tubes, the supernatant fluid decanted, and the precipitate washed at least 4 times in the same manner using 20 to 30 cc. portions of dilute ammonia for each washing.

Magnesia mixture was always added to insure complete precipitation of the phosphorus and the decanted fluid was allowed to stand 6 to 12 hours when any crystals of magnesium ammonium phosphate that had formed were removed by filtration. In all cases, before addition of the sodium reagent, the filtrate was evaporated to dryness and the residue dissolved in the least possible distilled water (usually 2 or 3 cc.). Samples taken from stool ash often contained a considerable quantity of ammonium salts. These were decomposed with nitric acid and evaporation continued to dryness.

Good results were obtained when samples of ash equivalent to 5 to 10 cc. of urine, 0.5 to 0.7 gms. of dried diet and 0.5 to 1.0 gm. of dried stool were used.

Potassium. The volumetric procedure described by Shohl and Bennett (20) for the determination of potassium as the iodoplatinate was used without alteration in these analyses.

Calcium. The gasometric method of VanSlyke and Sendroy (22) was slightly modified and used for the determination of calcium. Although a micromethod, designed for the determination of blood calcium, it was found applicable to the determination of calcium in urine, stool, and diet.

[1] May be obtained from Will Corporation, Rochester, N.Y.

[2] Precautions must be taken to obtain sodium free reagents. The C. P. magnesium chloride used in the laboratory was found to contain so much sodium that it could not be used in the preparation of magnesia mixture for use in the sodium determinations. A satisfactory magnesium salt was made by extracting 50 to 60 gms. of magnesium oxide 4 or 5 times with boiling water and dissolving it in pure hydrochloric acid.

In this laboratory it has consistently given more accurate results than either the volumetric procedure described by McCrudden (15) or that described by Shohl and Pedley (21).

Samples of ash were so diluted that quantities of 2 to 4 cc. contained from 0.2 to 0.6 mgs. of calcium. The portions for analysis were accurately pipetted into 15 cc. centrifuge tubes and one or two drops of 1 per cent alcoholic solution of methyl red added as an indicator. If not already distinctly acid the solution was acidified by adding a drop or two of hydrochloric acid and the volume brought to approximately 6 cc. with distilled water. One cubic centimeter of a saturated solution of ammonium oxalate was now added to each tube and the contents of the tube thoroughly mixed with a glass rod. Enough dilute ammonia (1 to 4) was added drop by drop with constant stirring to change the color of the indicator from red to a faint pink (intermediate between pink and yellow). The rod was removed and washed with a few drops of distilled water, the washings being permitted to fall into the tube. The tubes were placed in a rack and allowed to stand 6 to 12 hours. The remainder of the analysis was carried out exactly as described by VanSlyke and Sendroy (22).

Iron. Abundant references to methods for the determination of iron in biological materials appear in the literature. Of many methods tried that which gave the best results was the electrometric titration of King and Washburne (12). King and Howard (11) have used this method for the determination of iron in small quantities of blood, and in our hands it has proved adaptable to iron in urine, stools, and diets.

The principle involved is the familiar one of reduction of ferric iron to ferrous by titration with titanous sulphate or chloride, determining the end point electrometrically (13). If a sufficiently dilute solution of titanous sulphate is used for the titration, very small amounts of iron can be determined with accuracy. For details as to the assembling of apparatus, preparation, preservation, and standardization of the titanous sulphate, determination of the end point, etc., the reader is referred to the papers by King and Washburne and King and Howard. Following is a brief resumé of the principal variations from the original procedure.

It was found that the titanous solution could be stored in an atmosphere of pure carbon dioxide prepared in a Kipp generator and washed through a solution of acid mercuric chloride. Commercial carbon dioxide and hydrogen in cylinders are not sufficiently pure.

The E.M.F. set up between the platinum and calomel electrodes immersed in the solution being titrated was measured by means of a "Student

Type" potentiometer, with an enclosed lamp and scale galvanometer, both made by Leeds and Northrup. The platinum electrode was made from a 32 gauge bright platinum wire, fused to a copper wire of the same dimensions, which served as a connection in place of mercury. The platinum was fused into the end of a small glass tube. The titration flask was immersed partially in a water bath at a temperature of 85–90° C. during the titration, and was kept in motion by a motor shaker attached to its neck.

The quantity of iron in the sample to be analyzed should not be less than 0.25 mg. nor more than 3 mg. when an approximately 0.002 N solution of titanous sulphate is used for its reduction. Ten grams of the dried diets, or one gram of dried fecal material was sufficient for a single determination. The quantity of iron found in urine was extremely small, as a rule, in agreement with the findings of other investigators (18) (10) (14). It ranged from a trace to 0.7 mg. per liter. The ash of 400 cc. of urine was used for each determination.

Preparation of material for analysis of Iron. When feasible all specimens were ashed by the dry method, and dissolved in dilute hydrochloric acid as previously described under methods of ashing. Nitric acid was avoided in preparation of ash for reasons mentioned under the heading "Precautions." Aliquot parts of the ash solution were pipetted into 100 cc. pyrex beakers, 2 or 3 cc. of sulphuric acid (sp.g. 1.8) were added and the water and hydrochloric acid present removed by evaporation on a steam bath. The sulphuric acid and salts which remained were dissolved as completely as possible by warming with about 25 cc. of 20 per cent sulphuric acid and transferred to the titration flask. Enough 20 per cent sulphuric acid was used in washing the beaker to bring the volume of liquid in the flask to approximately 50 cc. In some instances considerable quantities of calcium sulphate precipitated on addition of the sulphuric acid. No attempt to remove this precipitate was made since it did not interfere with the iron determination. The 20 per cent sulphuric acid used as solvent was prepared as was that used in making the titanous sulphate solution and was stored in an atmosphere of carbon dioxide. The titration was performed as directed by King and Washburne (12).

Precautions were taken against the introduction of iron or other metals capable of being reduced, either through the use of impure reagents or through metal containers. Very small amounts of copper do not interfere, since iron may be determined in the presence of minute amounts of copper by this method (13). When oxidizing agents such as nitric or perchloric

acids were used in the ashing process it was necessary to remove all traces of these acids or their disintegration products prior to titration. To accomplish this the pyrex beakers were heated cautiously on a hot plate, after the water had been removed by evaporation on a steam bath. If the residue remaining in the beaker was small when fumes of sulphur trioxide ceased to be evolved, it was cooled and dissolved in 20 per cent sulphuric acid, and, if a considerable residue remained, 2 or 3 cc. more concentrated sulfuric acid were added and the process repeated to insure that all salts other than phosphates present in the ash had been converted into sulphates before transfer into the titration flask. Since interference by other ash constituents was never encountered it was never found necessary to separate iron from them.

Recovery experiments were made to determine the accuracy with which small amounts of iron could be recovered after addition to dried stool or diet specimens. When 2 mgm. of iron were added to a specimen of feces containing 6 mgm. all was recovered except 0.001 mgm., a negative error of 0.5 per cent. When 1 mg. was added to a dried diet sample containing 1.5 mgm. it was recovered with a negative error of 1 per cent. Routine use of the method has shown that the percentage error may be greater than that indicated by the recovery experiments, and that it may amount to as much as ± 5 per cent.

Summary and Conclusions

This paper gives in detail the technique for a comprehensive study of the simultaneous exchanges of sodium, potassium, calcium, magnesium, iron, nitrogen, and phosphorus.

1. The organization and methods of a special metabolism ward are described.

2. A comparison is made of the results of analyzing an entire diet for a day and of calculating the total mineral intake from tables. The values found for calcium were consistently lower than the calculated, and for sodium consistently higher. The discrepancies in the case of nitrogen, phosphorus, potassium, magnesium, and iron were of smaller magnitude, within the limits of accuracy of the procedure as a whole.

3. The same diet menus, weighed out and analyzed in different years, showed surprisingly close agreement on analysis.

4. The methods of preparation of foods and excreta for sampling, ashing, and analysis are given in detail.

The analytical methods used were selected after many trials as those giving the best results with the materials of both foods and excreta.

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THE RELATIVE VALUES OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL IN THE NUTRITION OF GROWING RATS

By

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IN THE interpretation of the results of investigations on rats concerned with biological measurements of the nutritive value of foods and food products, it is generally stated or implied that, at least relatively, the values secured are applicable to other species of animals and to man. There would be little defense for such investigations, aside from their contributions to comparative zoology, if this implication were not substantially true, and yet there is very little direct evidence on the point.

The recent paper of Bethke and his associates (1) concerned with the comparative nutritive value of the proteins of linseed meal and cottonseed meal for different animals directly challenges this viewpoint. The experimental data obtained have been interpreted to mean that for rats and calves the two proteins are of nearly equal nutritive value, but that for chickens cottonseed meal proteins are quite distinctly more efficient than linseed meal proteins, while for pigs the toxicity of cottonseed meal, fed at a level that proved non-toxic for the other species, prevented a comparison of protein values.

Because of the importance of the question involved, and because of the fact that the evidence adduced by the Ohio investigators did not in all cases relate directly and unequivocally to the protein values of their experimental rations, it seemed desirable to repeat the comparisons of the two feeds, using strictly quantitative and identical methods for each species of animal studied. The present paper reports the results of the experiments with albino rats.

The Paired-Feeding Experiment

The linseed and cottonseed meals used in all of the experiments were ground fine, partially dried in a low temperature oven, and extracted with ether. The cottonseed meal thus prepared contained 5.24 per cent of

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moisture, 1.80 per cent of ether-soluble material, 10.94 per cent of crude fiber, and 7.09 per cent of nitrogen. The linseed meal contained 6.35 per cent of moisture, 0.27 per cent of fat, 10.09 per cent of fiber, and 6.31 per cent of nitrogen.

The meals were each embodied in a ration so as to furnish approximately 8 per cent of crude protein. The other constituents of the ration contributed minimal amounts of protein but adequate amounts of other known nutrients, so that each ration presumably was complete except for its protein content. The composition of the rations is given in Table I.

TABLE I

THE PERCENTAGE COMPOSITION OF THE EXPERIMENTAL RATIONS

Components	Cottonseed meal ration	Linseed meal ration
Ether extracted meal	18.05	20.29
Osborne and Mendel salts	4.00	4.00
Sucrose	10.00	10.00
Butterfat	7.72	8.00
Sodium chloride	1.00	1.00
Dried yeast	0.50	0.50
Cod liver oil	2.00	2.00
Starch	56.73	54.21
	100.00	100.00

The rations contained practically the same percentages of crude fiber and of fat. After drying at about 70° C. for 48 hours, they were analyzed. The cotton seed meal ration contained 1.349 per cent of nitrogen, and the linseed meal ration contained 1.394 per cent of nitrogen, equivalent respectively to 8.43 and 8.71 per cent of crude protein. In gross energy the rations contained 4435 and 4405 small calories per gram, respectively.

In the paired-feeding experiment, 8 pairs of rats were used. Each pair was of the same sex and from the same litter, and the pair mates did not differ in initial weight by more than 3 gms. The rats in each pair, one on the cottonseed ration and the other on the linseed ration, received the same amount of food, which was increased continuously until one or the other refused some of it. After reducing the amount offered until both rats cleaned it up, the daily food allowance was again increased. At the end of each week all rats were weighed, and all food residues were weighed and discarded. After the second week, each rat received daily two drops of tiki-tiki, prepared in the laboratory according to the directions of Wells (2). The feeding continued for 57 days.

The initial and final weights (averages of weights on three consecutive

days), the total gains, and the total food consumption of the rats are given in Table II. There is also included in this table the body lengths of the rats from tip of nose to anus, determined after killing the rats with ether.

In each of the 8 pairs of rats, the rat raised on the linseed meal ration exceeded its pair mate in total gain. Such a consistent outcome, favoring either one or the other ration, would have resulted from chance only, twice in 256 trials. The average of the 8 differences in total gain between pair mates is 8.4 gms., and their standard deviation is 3.3 gms. The average difference is more than 2.5 times the standard deviation. According to "Student's" probability tables (3), for $n = 8$ and $z = 2.5$ the odds are only 1 in about 10,000 that an average difference of this size or larger would have resulted by chance.

In Table III, a comparison of the weekly gains of pair mates is made. For each week and each pair of the experiment a sign is given to indicate which of the rats gained the faster, if their gains were different. Again the evidence is quite decisive that the linseed meal promoted a faster growth. In 41 comparisons the rat receiving the linseed ration gained the faster, in only 12 comparisons was the reverse true, while in 11 comparisons the gains of pair mates were equal.

TABLE II

SUMMARY OF BODY WEIGHTS, GAINS, FEED RECORDS, AND BODY LENGTHS

	Pair 1♂		Pair 2♂		Pair 3♂		Pair 4♀		Pair 5♂		Pair 6♂		Pair 7♀		Pair 8♀	
	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal	Cotton-seed meal	Lin-seed meal
Initial weight, gms.	54	52	47	47	52	51	51	54	63	61	49	51	52	54	49	49
Final weight, gms.	88	96	82	91	95	103	92	108	115	115	89	97	96	104	88	100
Gains, gms.	34	44	35	44	43	52	41	54	52	54	40	46	44	50	39	51
Total food, gms.	326	328	339	342	400	400	395	395	406	400	332	331	381	377	351	352
Body length, cms.	16.7	17.5	16.5	17.0	17.1	17.3	16.9	17.7	17.9	18.2	17.1	17.1	17.1	17.4	16.3	16.9

TABLE III

A COMPARISON OF THE WEEKLY GAINS MADE BY PAIR MATES

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	−	±
1	+	±	±	±	±	+	±	+	3	0	5
2	+	+	+	+	±	+	+	+	7	0	1
3	±	±	−	+	+	−	+	+	4	2	2
4	+	+	+	±	−	+	+	+	6	1	1
5	−	+	−	−	+	±	+	+	4	3	1
6	+	−	+	±	−	+	+	+	5	2	1
7	+	+	+	+	−	−	−	+	5	3	0
8	+	+	+	+	+	+	+	−	7	1	0
	Totals per pair								Totals for experiment		
+	6	5	5	4	3	5	6	7	41		
−	1	1	2	1	3	2	1	1		12	
±	1	2	1	3	2	1	1	0			11

\+ indicates a greater weekly gain by the rat receiving the linseed meal ration.
− indicates a greater weekly gain by the rat receiving the cottonseed meal ration.
± indicates equal weekly gains by pair mates.

For 61 weekly comparisons, excluding 3 whose significance was somewhat discounted by appreciable differences in the weekly consumption of food, the average difference in gain between pair mates was .93 ± .13.

Referring again to Table II, it will be noted that the body length of the rats on the linseed meal ration exceeded that of the rats on the cottonseed meal ration in seven pairs, while in one pair (No. 6) the pair mates were of equal body length. The average difference was 4.4 mm. and the standard deviation of the 8 differences 2.6 mm., z thus being 1.7. The odds are only 1 in over 700 that as great or greater difference between pair mates would have been obtained by chance only. This evidence establishes the conclusion that the more rapid gains induced by the linseed meal ration over the cottonseed meal ration, when the amounts consumed were equalized, was due to a more rapid growth rather than to a more rapid fattening.

THE NITROGEN METABOLISM EXPERIMENT

The same two rations were used in a nitrogen balance experiment on 5 rats to determine the biological values of linseed meal and cottonseed meal proteins by Mitchell's modification (4) of Thomas' method. The rats were litter mates, 4 being females and 1 a male. Their initial age was about 7 weeks and their weights ranged from 51 to 61 grams.

The standardizing ration used in the initial and final periods had as its source of protein whole dried ether-extracted egg and contained 4.54 per cent of crude protein. It was made up of 5.02 per cent of the egg preparation, 4 per cent of Osborne and Mendel's salt mixture, 8 per cent of butterfat, 1.90 per cent of agar, 10 per cent of sucrose, 1 per cent of NaCl, 0.5 per cent of dried yeast, 2 per cent of cod liver oil, and 67.58 per cent of corn starch.

The collection periods were from 6 to 9 days in length, and were in each case preceded by at least 4 days of preliminary feeding on the same ration. In order to improve the consumption of the experimental rations, three drops of tiki-tiki were given to each rat daily after the first week, and in the final two periods the feed was mixed, in the individual feed cup, with hot water, instead of cold water, partially to dextrinize the starch. After the latter treatment there was less food refused by the rats. In the next to the last transition period, each rat was given daily 0.5 gm. of Northwestern Yeast Co.'s dried yeast up to within one day of the start of the following collection period, in a further attempt to stimulate food consumption.

The apparatus used was developed in Mitchell's laboratory for this type of work, and is the same as that already described in published articles except that the large crystallizing dishes containing the rats are covered, except for a small space around the edge, by large inverted glass funnels connected by rubber tubing to a suction pump. The pump affords effective ventilation for the metabolism dishes.

The results of the initial and final standardizing periods are given in Table IV. The purpose of these periods is to determine the excretion of metabolic nitrogen in the feces, per gram of food consumed, and of endogenous nitrogen in the urine, per 100 grams body weight. These factors differ in the two periods for each rat, and in computing the biological values for the two intervening periods, they are presumed to change in a linear fashion from the initial to the final value determined. In the computations of the intermediate periods, these factors are used in estimating the contribution of the body of the rat to the nitrogen appearing in the feces and in the urine.

In the second experimental period Rats 1, 2, and 3 received the cottonseed meal ration, and Rats 4 and 5 the linseed meal ration, while in the third period the rations were reversed for these two groups of rats. In Table V, the results have been assembled for the two rations.

The biological values obtained for the cottonseed meal proteins ranged from 75 to 80 and averaged 78. Those obtained for the linseed meal pro-

TABLE IV

THE RESULTS OF THE INITIAL AND FINAL STANDARDIZING PERIODS, EXPRESSED ON THE DAILY BASIS

Animal Number	Initial weight gms.	Final weight gms.	Average weight gms.	Food intake gms.	Fecal nitrogen mgms.	Body nitrogen in feces: Per gm. of food mgms.	Urinary nitrogen mgms.	Body nitrogen in urine: Per 100 gms. weight mgms.
Initial period, dried egg protein ration, containing .727 per cent N								
1♀	50	52	51.3	5.2	7.73	1.49	19.00	37.04
2♂	54	55	55.0	4.6	8.35	1.82	15.82	28.76
3♀	53	55	54.3	5.5	9.71	1.77	22.68	41.77
4♀	52	54	53.5	5.2	8.79	1.69	19.60	36.64
5♀	57	63	60.3	5.8	8.38	1.44	16.60	27.53
Final period, dried egg protein ration, containing .727 per cent N								
1♀	78	81	79.8	6.5	17.42	2.68	27.54	34.51
2♂	78	83	80.5	6.5	14.07	2.16	21.74	27.01
3♀	82	85	83.8	6.5	13.07	2.01	26.20	31.26
4♀	82	85	83.8	6.5	12.93	1.99	26.34	31.43
5♀	91	95	93.0	6.5	14.21	2.19	28.44	30.58

TABLE V

THE BIOLOGICAL VALUES OF COTTONSEED MEAL AND LINSEED MEAL PROTEINS, RESULTS EXPRESSED ON THE DAILY BASIS

							Body N in feces					Body N in urine				
Animal Number	Initial wt. gms.	Final wt. gms.	Av. wt. gms.	Food intake gms.	N intake mgms.	Fecal N mgms.	Per gm. of food mgms.	Per day mgms.	Food N in feces mgms.	Absorbed N mgms.	Urinary N mgms.	Per 100 gms. wt. mgms.	Per day mgms.	Food N in urine mgms.	Food N retained mgms.	Biol. value per cent
Cottonseed meal ration containing 1.349 per cent N																
1♀	59	65	62.0	5.94	80.13	26.99	1.89	11.23	15.76	64.37	36.28	36.20	22.44	13.84	50.53	78
2♂	64	67	65.8	5.45	73.52	25.98	1.93	10.52	15.46	58.06	32.78	28.18	18.54	14.24	43.82	75
3♀	60	66	63.3	6.20	83.64	27.64	1.85	11.47	16.17	67.47	39.56	38.27	24.22	15.34	52.13	77
4♀	78	82	80.3	6.50	87.69	31.34	1.89	12.29	19.05	68.64	40.34	33.17	26.64	13.70	54.94	80
5♀	85	91	88.3	6.50	87.69	28.29	1.94	12.61	15.68	72.01	40.90	29.56	26.10	14.80	57.21	79
															Average	78
Linseed meal ration containing 1.394 per cent N																
1♀	72	79	75.8	6.50	90.61	23.70	2.28	14.82	8.88	81.73	42.52	35.35	26.80	15.72	66.01	81
2♂	77	75	76.5	4.70	65.52	17.35	2.05	9.64	7.71	57.81	34.10	27.59	21.11	12.99	44.82	78
3♀	78	82	80.3	6.49	90.47	20.76	1.93	12.53	8.23	82.24	45.58	34.76	27.91	17.67	64.57	79
4♀	64	70	67.0	6.07	84.62	19.78	1.79	10.87	8.91	75.71	41.48	34.90	23.38	18.10	57.61	76
5♀	66	74	70.5	5.81	80.99	18.71	1.69	9.82	8.89	72.10	39.74	28.55	20.13	19.61	52.49	73
															Average	78

TABLE IV

THE RESULTS OF THE INITIAL AND FINAL STANDARDIZING PERIODS, EXPRESSED ON THE DAILY BASIS

Animal Number	Initial weight gms.	Final weight gms.	Average weight gms.	Food intake gms.	Fecal nitrogen mgms.	Body nitrogen in feces: Per gm. of food mgms.	Urinary nitrogen mgms.	Body nitrogen in urine: Per 100 gms. weight mgms.
Initial period, dried egg protein ration, containing .727 per cent N								
1♀	50	52	51.3	5.2	7.73	1.49	19.00	37.04
2♂	54	55	55.0	4.6	8.35	1.82	15.82	28.76
3♀	53	55	54.3	5.5	9.71	1.77	22.68	41.77
4♀	52	54	53.5	5.2	8.79	1.69	19.60	36.64
5♀	57	63	60.3	5.8	8.38	1.44	16.60	27.53
Final period, dried egg protein ration, containing .727 per cent N								
1♀	78	81	79.8	6.5	17.42	2.68	27.54	34.51
2♂	78	83	80.5	6.5	14.07	2.16	21.74	27.01
3♀	82	85	83.8	6.5	13.07	2.01	26.20	31.26
4♀	82	85	83.8	6.5	12.93	1.99	26.34	31.43
5♀	91	95	93.0	6.5	14.21	2.19	28.44	30.58

TABLE V

THE BIOLOGICAL VALUES OF COTTONSEED MEAL AND LINSEED MEAL PROTEINS, RESULTS EXPRESSED ON THE DAILY BASIS

Animal Number	Initial wt. gms.	Final wt. gms.	Av. wt. gms.	Food intake gms.	N intake mgms.	Fecal N mgms.	Body N in feces: Per gm. of food mgms.	Body N in feces: Per day mgms.	Food N in feces mgms.	Absorbed N mgms.	Urinary N mgms.	Body N in urine: Per 100 gms. wt. mgms.	Body N in urine: Per day mgms.	Food N in urine mgms.	Food N retained mgms.	Biol. value per cent
Cottonseed meal ration containing 1.349 per cent N																
1♀	59	65	62.0	5.94	80.13	26.99	1.89	11.23	15.76	64.37	36.28	36.20	22.44	13.84	50.53	78
2♂	64	67	65.8	5.45	73.52	25.98	1.93	10.52	15.46	58.06	32.78	28.18	18.54	14.24	43.82	75
3♀	60	66	63.3	6.20	83.64	27.64	1.85	11.47	16.17	67.47	39.56	38.27	24.22	15.34	52.13	77
4♀	78	82	80.3	6.50	87.69	31.34	1.89	12.29	19.05	68.64	40.34	33.17	26.64	13.70	54.94	80
5♀	85	91	88.3	6.50	87.69	28.29	1.94	12.61	15.68	72.01	40.90	29.56	26.10	14.80	57.21	79
															Average	78
Linseed meal ration containing 1.394 per cent N																
1♀	72	79	75.8	6.50	90.61	23.70	2.28	14.82	8.88	81.73	42.52	35.35	26.80	15.72	66.01	81
2♂	77	75	76.5	4.70	65.52	17.35	2.05	9.64	7.71	57.81	34.10	27.59	21.11	12.99	44.82	78
3♀	78	82	80.3	6.49	90.47	20.76	1.93	12.53	8.23	82.24	45.58	34.76	27.91	17.67	64.57	79
4♀	64	70	67.0	6.07	84.62	19.78	1.79	10.87	8.91	75.71	41.48	34.90	23.38	18.10	57.61	76
5♀	66	74	70.5	5.81	80.99	18.71	1.69	9.82	8.89	72.10	39.74	28.55	20.13	19.61	52.49	73
															Average	78

teins varied somewhat more, from 73 to 81, but averaged the same, *i.e.*, 78. In each case, apparently, an average of 78 per cent of the absorbed nitrogen of these meals was retained in the bodies of the growing rats, for the replacement of the endogenous losses of body nitrogen in maintenance and for the construction of new tissue in growth.

There is thus no difference in the utilization of the absorbed nitrogen of the two meals by growing rats, although in the paired-feeding experiment the linseed meal ration was distinctly superior in the promotion of growth. In searching the metabolism data of Table V for some clue to account for this conclusive superiority of the linseed meal ration in the promotion of growth, it appears that the protein (nitrogen) in this ration was digested to a distinctly better extent than the protein in the cottonseed meal ration. In Table VI, the computed coefficients of apparent and of true digestibility

TABLE VI

THE DIGESTIBILITY OF THE NITROGEN IN THE LINSEED MEAL AND COTTONSEED MEAL RATIONS

Rat No.	Linseed meal ration		Cottonseed meal ration	
	Apparent digestibility %	True digestibility %	Apparent digestibility %	True digestibility %
1	73.8	90.2	66.3	80.3
2	73.5	88.2	64.7	79.0
3	77.0	90.9	66.9	80.7
4	76.6	89.5	64.3	78.3
5	76.9	89.0	67.7	82.1
Average	75.6	89.6	66.0	80.1

of the nitrogen are summarized. The coefficients of apparent digestibility have been computed from the total fecal nitrogen excretion, while the coefficients of true digestibility have been computed from the estimates of absorbed nitrogen in Table V.

Apparently, on either basis, the linseed meal nitrogen was about 9 per cent more digestible than the cottonseed meal nitrogen, a difference that might account for the greater growth-promoting value of the linseed meal ration.

However, it is possible that the digestibility of the non-protein constituents of the two rations also varied. To test this possibility the two rations were fed in turn to four mature rats and the feces were collected, dried, weighed, and analyzed for nitrogen and for gross energy in the bomb

calorimeter. The collection periods were of 7 days duration, preceded by 4-day preliminary periods. The results of this test are summarized in Table VII. Here again, a distinct difference in the digestibility of nitrogen is evident, but the gross energy of the rations was about equally well utilized *i.e.*, to the extent of 91 to 92.

It may be concluded, therefore, that the superiority of the linseed meal ration in the promotion of growth was entirely due to the greater digestibility of its nitrogen.

The biological values obtained for cottonseed meal proteins and linseed meal proteins in these experiments, averaging 78 in each case, are higher than those reported by Bethke and his associates (1), *i.e.*, 72 and 71, respectively. This is probably due to the fact that in the Ohio experiments the proteins were fed at a higher level, 10 per cent as compared with 8 per cent.[1] Mitchell (5) has shown that the biological value of a protein decreases as its concentration in the experimental ration increases. Bethke and co-workers did not obtain the marked difference in digestibility of cottonseed meal and linseed meal nitrogen noted in these experiments. For the true digestibility of nitrogen in the cottonseed meal ration they obtained an average coefficient of 83.6, and for the linseed meal ration an average of 85.7.

Nevens (6) in this laboratory has reported the results of determinations of the biological value of cottonseed meal proteins for rats in rations containing 10 per cent of protein. Two trials were made on each of 3 rats. He obtained an average biological value of 66, and an average true digestibility of 74 per cent; the apparent digestibility averaged 61 per cent.

Summary and Conclusions

In paired-feeding experiments on white rats, a ration containing approximately 8 per cent of linseed meal proteins proved definitely superior in growth-promoting value to a ration similar in every respect except for the substitution of linseed meal protein for cottonseed meal protein. The former ration induced more rapid gains in body weight on the same amounts of food and more rapid growth in length of body. Eight pairs of rats were used in this test.

In nitrogen balance studies on five rats, the biological values of the

[1] It should also be noted that in these experiments the rations of all periods contained 6 per cent of yeast, supplying to the ration 2.5 to 3.0 per cent of protein above the 10 per cent furnished by the cottonseed and linseed meals. This constitutes in several respects a departure from the method used in the experiments under discussion, the effect of which on the biological values cannot be evaluated.

TABLE VII

THE UTILIZATION OF NITROGEN AND OF ENERGY IN THE LINSEED MEAL AND THE COTTON SEED MEAL RATION,*
RESULTS EXPRESSED ON THE WEEKLY BASIS

Rat	Body weight gms.	Food intake gms.	Nitrogen intake gms.	Energy intake cals.	Weight dry feces gms.	Nitrogen in feces %	Fecal nitrogen gms.	Digestibility of N %	Energy per gm. feces cals.	Fecal energy cals.	Digestibility of energy %
Linseed meal ration 1.394 per cent N 4405 cals. per gram.											
1♂	266	70	.9578	308	7.1990	3.70	.2664	72.7	4.002	28.8	90.6
2♂	285	70	"	"	6.8682	3.59	.2466	74.7	4.028	27.7	91.0
3♂	242	70	"	"	7.0264	3.60	.2530	74.1	4.173	29.3	90.5
4♂	297	70	"	"	6.6804	3.72	.2485	74.5	3.875	25.9	91.6
							Average	74.0			90.9
Cottonseed meal ration 1.349 per cent N 4435 cals. per gm.											
1♂	273	70	.9443	310	6.3686	4.87	.3102	67.1	3.988	25.4	91.8
2♂	288	70	"	"	6.3904	4.73	.3023	68.0	4.026	25.7	91.7
3♂	250	70	"	"	6.2501	4.87	.3044	67.8	4.106	25.7	91.7
4♂	297	70	"	"	6.3194	4.71	.2976	68.5	4.042	25.5	91.8
							Average	67.9			91.7

* Grateful acknowledgment is made to Mr. Frank Simpson, of the Division of Animal Nutrition, for performing this test of the experimental rations.

nitrogen in the two experimental rations were found to be the same, averaging 78 in each case. However, the digestibility of the cottonseed meal nitrogen was 9 per cent less than that of the linseed meal nitrogen. The gross energy of the two rations was shown to be equally digestible, so that the superior growth-promoting value of the linseed meal ration must have been due solely to its greater content of digestible protein.

It may be concluded that the biological values of linseed meal proteins and cottonseed meal proteins are the same for rats, but that their digestibilities may differ markedly, depending probably upon a variable inclusion of hulls in cottonseed meal.

Grateful acknowledgement is here made to Dr. H. H. Mitchell, head of the Division of Animal Nutrition, who proposed this study and supervised it throughout, giving kindly suggestions and criticisms.

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THE AGE FACTOR IN THE RESPONSE OF THE RAT TO LEVEL OF DIETARY PROTEIN*

By

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DURING the past decade much has been written about the deleterious effect of diets rich in protein. Since the kidney is the organ chiefly concerned with the removal of nitrogenous products in the metabolism of protein, attention has naturally been focused on this organ. However, in view of the apparent simplicity of the thesis, the experimental results have been surprisingly variable. It seems, therefore, that any controlled observations of the effects of high concentrations of dietary protein will be valuable in helping to clarify a situation which involves a subject of considerable practical importance.

Among other confusing statements in the literature are those concerning growth and physiological well-being of experimental animals on rations rich in protein. In some cases poor growth is reported; in others, satisfactory early growth but failure to reach the usual mature body weight; while normal development is observed in still other studies. In view of the lack of concordance in this respect and in the light of recent accounts (1, 2, 3) of rapid growth of albino rats—the animal most frequently used in these experiments—it seemed desirable to examine anew the influence of dietary protein upon the development of individuals of the same strain of rapidly-growing animals. The increase in body weight in young animals and maintenance in old ones should be a reliable criterion of the adequacy of a diet under conditions where other variables are ruled out. In the present report we have attempted to correlate change in body weight with concentration of dietary protein in white rats of different ages.

The observations were made upon a large number of male albino rats from all of which the right kidney had been removed. They were grouped into four different ages and were given complete diets containing three different amounts of protein (see Table I). At 56 days and 150 days after nephrectomy (the experimental ration was first given on the day of the

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operation) rats from each age group were killed and observations and organ measurements made. The data were used only from those animals which at

TABLE I
COMPOSITION OF EXPERIMENTAL RATIONS

Food material	Diet 18	Diet 60	Diet 85	
Casein	18%	60%	85%	700 mgm. dried yeast daily given apart from basal ration. Casein contained 12.1% N, 8.2% moisture, 5.2% ash. For preparation of salt mixture see Osborne, T. B. and Mendel, L. B., *Jour. Biol. Chem.*, 1917, XXXII, 374.
Hydrogenated veg. fat*	22	22	8	
Cod liver oil	5	5	4	
Corn starch	51	9		
Salts	4	4	3	
Energy value per gm.	5.2 Cal.	5 Cal.	4.2 Cal.	
Protein calories	12%	38%	67%	

* Crisco.

autopsy had no evidence of pulmonary infection, and are summarized in Table II.

It is seen that all the animals which were 30 days old at the beginning of the experiment showed very satisfactory growth both for 56 days and for 150 days. It has previously been shown (4, 5) that unilateral nephrectomy *per se* is attended with no demonstrable alteration in rate of growth when the diet contains fairly low concentrations of protein. In the present study, however, even on the ration containing moderately high concentration of protein (Diet 60) the growth over a period of 56 days compares favorably with the rapid growth records recorded in the recent literature (1, 2, 3). Within this age group (30 days), however, it is obvious that the gain in weight over 56 days and over 150 days period is distinctly less on the very high-protein ration than on the other diets. Like observations were made by Smith and Jones (4) on young rats given similar rations for a much longer time. It is interesting to note that the ratio of total calories ingested to grams of gain is almost indentical on all three diets. The favorable responses of the group on Diet 60 and the group on Diet 85 over the period of most rapid growth, together with the poorer behavior of older animals on Diet 85, seem to confirm the principle emphasized by Burr and Burr (6) that the nutritive ratio should be relatively high during rapid growth and lower as it proceeds beyond this period.

In the group 90 days old at the beginning of the experiment there is naturally less increase in body weight, but the relative gains on the Diet 60

TABLE II

Ration No.	Age at beginning	Duration	Number of rats	Average body wt. at beginning	Average final body wt.	Per Cent change in body wt.	Total calories consumed	Total Calories per gm. gain in body wt.	Average gain per day
	days	days		grams	grams				grams
18	30	56	9	77	282	273± 60.2	2841	13.9	3.7
60	30	56	8	80	347	333± 47.9	3253	12.2	4.8
85	30	56	10	74	249	238± 53.5	2404	13.7	3.1
18	30	150	12	65	468	619± 90.6	9416	23.4	2.7
60	30	150	11	68	413	519± 95.4	8105	23.5	2.3
85	30	150	12	77	403	445±133.8	7899	24.2	2.2
18	90	56	14	274	408	53± 16.5	3593		
60	90	56	12	228	347	53± 9.5	2915		
85	90	56	14	280	351	22± 4.6	2822		
18	90	150	6	253	441	74± 12.1	8736		
60	90	150	8	251	461	85± 16.6	9445		
85	90	150	9	293	384	30± 7.1	7480		
18	180	56	14	431	460	5± 7.0	3240		
60	180	56	12	355	414	16± 7.6	3060		
85	180	56	16	396	390	−1± 5.0	2835		
18	180	150	17	399	492	27± 17.6	8674		
60	180	150	11	401	457	14± 8.0	7715		
85	180	150	16	378	394	4± 6.8	7640		
18	360	56	8	513	536	5± 4.7	3645		
60	360	56	3	435	455	5± 4.7	3405		
85	360	56	11	516	446	−13± 8.6	2944		
18	360	150	8	491	537	8± 13.9	9651		
60	360	150	8	442	494	6± 5.9	9455		
85	360	150	12	480	435	−10± 6.8	7724		
LAPAROTOMY									
18	90	56	5	274	418	53± 8.3	3650		
60	90	56	6	251	370	49± 11.1	2730		
85	90	56	4	307	404	32± 8.0	3389		
18	90	150	7	263	465	84± 35.9	9625		
60	90	150	4	252	410	72± 12.0	7580		
85	90	150	6	274	336	35± 4.7	7686		

equal or exceed those on the low protein ration. While the animals to which Diet 85 was given did not grow so well as those on the other two rations, they were in excellent physical condition at autopsy. In a small group of operative control rats 90 days old, the relative change in body weights of the groups on the three diets is similar to the animals with one kidney and again shows the favorable effect of a ration moderately rich in protein, as well as the retarding influence of very high concentrations of dietary protein.

As might be expected, the data for the rats 180 days old show smaller relative gains in weight. Here again the diet containing the very high concentration of protein permits the least gain though the animals in this group were in good condition after 150 days.

The rats 360 days old at the beginning of the experiment gained little. Even in this group Diet 60 compares favorably with the low protein ration after 150 days. The animals on Diet 85 lost weight and showed noticeably less subcutaneous and intra-abdominal fat at autopsy than did those on Diets 18 or 60. The data presented by Jackson and Moore (7) show essentially the same unfavorable change in body weight of their older rats with one kidney, compared to younger animals similarly treated, when all were given a diet relatively rich in protein over periods varying from 2 to 15 months. The largest individual final body weight in the group on Diet 85 was Rat 834 which weighed 496 grams after losing 30 grams in the course of 150 days; on Diet 18, Rat 1011 which weighed 703 grams after 150 days; and on Diet 60, Rat 1097 weighing 600 grams after 150 days.

The energy intake of the rats with one kidney is less on the very high protein diet than on the other rations in all the age groups, which fact would explain the failure to make the expected gains in body weight. There seems little room for doubt that this decreased food consumption is associated with some circumstance of the metabolism of the large quantities of protein ingested. The fact that the blood urea concentrations are markedly higher in the rats with one kidney on Diet 85 than on the other rations appears to substantiate this conclusion, although urea itself might not be the causative agent, for it has been shown that enlargement of the kidneys and renal damage resulting from feeding rations rich in protein do not occur when an equivalent amount of excess nitrogen is given as urea (8). That this is probably not the only explanation is suggested by the operative control group in which the animals on Diet 85 consumed more energy than the rats on Diet 60, yet made less gain in weight. However, the numbers of animals are too small for definite conclusions to be drawn in these latter

groups. That the rations were adequate from the qualitative point of view was shown by complete absence of symptoms or lesions attributed to a deficiency of accessory food factors or salts.

On the basis of the data collected in the present study it appears that under the conditions imposed by unilateral nephrectomy, maintenance, growth and general well-being are promoted in rats of widely different ages when the ration contains 38 per cent of the calories as protein, as well as or better than when only 12 per cent are derived from this foodstuff. Furthermore, good, though not maximal, rates of growth are supported in young rats 30 days of age at the beginning of the feeding period on a ration, 67 per cent of the total calories of which arises from its protein content. This ration, however, is not favorable for maintenance or growth of rats 6 and 12 months old over periods of 56 or 150 days.

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FAT SOLUBLE VITAMINS

XXXII. THE DISTRIBUTION OF VITAMIN A IN TOMATO AND THE STABILITY OF ADDED VITAMIN D*

By

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THERE are two elements of interest in modern studies on the occurrence and distribution of vitamins. One is founded on the intensely practical objective of rationalizing the process of compounding a satisfactory ration. The other is founded on the desire to learn more about the nature of vitamins and to elucidate the mysteries of their function in the metabolism of both plants and animals. These combined prospects should certainly furnish enough inducement for the careful study of the vitamins of food materials especially those which are particularly rich in one or more of them. In this paper, we have chosen to give consideration to the vitamins of tomatoes.

HISTORICAL

The vitamin content of tomatoes has attracted attention ever since Givens and McCluggage (1) and Hess and Unger (2) in 1918 pointed out that tomato is a potent source of the antiscorbutic vitamin. The former found that 10 gms. of fresh raw tomato daily protected guinea pigs from scurvy, and when scurvy was already incident this amount cured the disease. They found that even dried tomatoes prepared at 55 to 60° with an exposure of 14 to 24 hours contained some vitamin C. Hess and Unger secured protection of guinea pigs from scurvy with 5 cc. of tomatoes which had been stored in a canned condition for almost a year. They thereupon fed canned tomatoes in amounts of 7.5 to 15 cc. to infants with good results. This led them to recommend canned tomato in place of the more expensive orange juice as an antiscorbutic.

In 1920 Hess (3) recommended canned tomato unreservedly for infant feeding in daily doses of one ounce. Hess and Unger (4, 5) reported that 5 cc. of strained canned tomato per day were necessary to protect guinea pigs from scurvy; 3 cc. were found insufficient. In view of the fact that vitamin C is generally less stable under alkaline conditions, they made tomato juice alkaline

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just before feeding, but observed no decreased potency. Boiled canned tomato juice was found somewhat less efficient.

Remy (6) found tomatoes sterilized by heat to contain considerable amounts of vitamin C, even after storage from 1 to 7 months. Givens and McCluggage (7, 8) found that a daily supplement of 2.5 gms. or even 1 gm. of fresh raw tomatoes would protect a guinea pig from scurvy. Heat processed tomatoes varied in their protective action. Tomatoes canned by the usual method of processing with 5 pounds steam pressure for 10 minutes or even 15 pounds for 30 minutes in daily doses of 10 gms. prevented scurvy in guinea pigs. Tomatoes dried at 35–40° C. for 52 hours were protective when fed in daily doses of .5 gm. La Mer, Campbell, and Sherman (9) studied the time curve of destruction of vitamin C by boiling filtered canned tomato juice. At the natural acidity of pH 4.2, one hour of boiling destroyed 50 per cent of the vitamin; at a reduced acidity of 5.1 the destruction increased to 58 per cent, and with a further reduction of acidity to a pH of 8.3 to 10.3, one hour of boiling destroyed 61 to 65 per cent. Three cc. of untreated canned tomato were found to give complete protection to a guinea pig.

House, Nelson, and Haber (10) found green tomatoes relatively poor in vitamin C. Air ripened and ethylene ripened tomatoes were richer in this vitamin than the green fruit, and vine ripened tomatoes were found superior to either the artificially ripened or the green tomatoes. The commercial method of ripening tomatoes in an ethylene air mixture was found to produce fruit equally as rich in vitamin C as fruit picked green and ripened in the air. Jones and Nelson (11) found the vitamin C content of tomatoes to increase as the fruit developed into a mature ripened condition. Naturally ripened tomatoes contained the most vitamin C. Ethylene ripened tomatoes contained no more vitamin C than the green fruit from which they had been prepared.

The latest report on the vitamin C content of tomatoes relative to its production and destruction is that by Clow and Marlatt (12). They found that there was an increase in vitamin C content with maturation of the fruit. Greenhouse tomatoes were found somewhat inferior but both greenhouse and field tomatoes picked green and then allowed to ripen at room temperature proved to be as potent as those ripened on the vine. Ripening with ethylene did not appear to prevent the usual development of vitamin C. Ripe tomatoes canned by the cold pack method were found as rich in vitamin C, nine months after storage, as freshly picked ripe tomatoes. However, after 15 to 20 months storage there were indications of some destruction. Green pickled tomatoes contained but little vitamin C. Of field ripened tomatoes 3 cc. daily were found sufficient to cure scurvy in a guinea pig.

That tomatoes contain vitamin B has also been known for some time. Hess and Unger (13) in 1919 found that pigeons could be cured of polyneuritis by the administration of 5 cc. canned tomato daily. Osborne and Mendel (14) in the same year included tomatoes in the list of fruits and vegetables in which vitamin B had been found to be present by experimental trial with rats. In 1920 Osborne and Mendel (15) found canned tomatoes dried at 60–70° C. rich in water-soluble vitamin B. One gm. daily doses promoted good growth. Five tenths gm. was not so effective, but even .2 gm. occasionally promoted limited growth. Osborne (16) has stated that tomatoes are among the richest of the vegetables on the dry basis in vitamin B, with spinach a close second.

Sherman and Burton (17) found that vitamin B in filtered tomato juice was more easily destroyed if made alkaline or even when the acidity was merely slightly decreased. The increased destruction upon heating for one hour at 100° at a pH of 7.9 instead of the natural pH of 4.28 was 20 to 30 per cent; at 9.2, 60 to 70 per cent; and at 10.9, 90 to 100 per cent. Oxygen did not appear to play a prominent part in the destruction. House, Nelson, and Haber (10) found that green tomatoes compared with ripe tomatoes were equally rich in vitamin B, and that ethylene treatment had no effect. It should, however, be mentioned that they observed some superiority in the ripened fruit, which was not regarded as of special significance. Jones and Nelson (11) found naturally ripened tomatoes a better source of vitamin B than any others, whether treated or untreated with ethylene. In a second series of experiments they obtained lower values for vitamin B

with the same fruit which had in the meantime been kept in storage. It suggested to them a partial destruction of the vitamin B with storage, but no definite conclusions were drawn.

That cod liver oil (18) butter fat (19, 20) and the green leafy parts of plants (21) are rich in vitamin A, and that this vitamin occurs abundantly in nature associated with yellow pigments (22, 23) of the carotinoid type has been known for some time. As a matter of fact, Euler and Hellström (24) found .005 mg. carotin to produce growth in rats when they were provided with vitamin D. Lycopin, the red isomer of carotin in tomato, was found inactive. Duliere, Morton and Drummond (25) however, found carotin inactive when highly purified. Moore (26) in turn has criticized the work of the later, and supported v. Euler and co-worker in their claims.

Whatever the facts may be in regard to carotin as to its serving in the capacity of vitamin A, or even its identity with vitamin A, carotin has long been known to be present in tomatoes (27); and tomatoes were found rich in vitamin A as early as 1920. At that time, Osborne and Mendel (28) found .1 gm. of dried tomatoes efficacious in supplying sufficient vitamin A to rats (29). Sherman and Munsell (30) found that .11 gm. of tomato daily, reinitiated growth in rats on a vitamin A-free diet. Of this tomato .17 gm. contained 1 rat unit of vitamin A as defined by them. Davis and Stillman (31) after carrying out experiments on rats suggested that tomatoes, among other fruits, could be used instead of orange juice to supply vitamin A to infants. Kohman (32) reported tomatoes 40 times as rich in vitamin A as strawberries. Sherman, Quinn, Day, and Miller (33) studied the stability to heat of vitamin A in tomato juice filtered free from suspended material in the same manner in which they studied the stability of vitamin B in this medium. When heated for 4 hours at 97°, 17 per cent of the vitamin A was found destroyed. An oxygen-free atmosphere did not reduce the destruction, nor did bubbling of air through the juice increase it. Changing the pH from 4.2 to 9.2 did not increase the destruction by heating in an atmosphere of nitrogen. Morgan and Smith (34) discovered the relative poverty of green tomatoes as compared with ripe tomatoes in vitamin A and its consistent development with the process of ripening. They pointed out that this was unexpected in view of the claims of Dye, Medlock, and Crist (35) that vitamin A and chlorophyll are produced simultaneously. They rather observed a simultaneous development of lycopin and vitamin A. Vine-ripened tomatoes and those ripened off the vines under various light conditions appeared to be equally rich in vitamin A. The adequacy of the ethylene ripening process was indicated. House, Nelson, and Haber (10) by feeding rats 2 gms. of tomatoes daily found green tomatoes lower in vitamin A content than ripe ones. With the latter the method of ripening did not appear to be a factor. Jones and Nelson (11) found naturally ripened tomatoes a better source of A than any other studied, including those ripened in ethylene. Green tomatoes did not vary in vitamin A content with stage of development or with ethylene treatment.

From the preceding review of the literature it can be appreciated that tomato is a potent source of vitamins A, B, and C. Hess (3) in 1920 when discussing tomato as a source of vitamin C, made the following statement, "As they have been shown by Osborne and Mendel to be rich in the water-soluble and the fat-soluble vitamins, canned tomatoes may be regarded from a nutritional standpoint as a palatable solution of the three vitamins." Morgan and Field (36) however, have reported that apricots compare favorably with the best figures reported for spinach, egg yolk, or butter. Peaches and prunes had less vitamin A than the apricots but as much or more than tomatoes, bananas, or lettuce. In our experiments we have found tomatoes from 1/5 to 1/10 as rich in vitamin A as a sample of good June butter.

Experimental

Vitamin A

In 1921, during the course of our studies on the relation of pigment to vitamin A, we had occasion to test tomatoes for their vitamin A content.

Fresh tomatoes were dried at a temperature approximating 100° until they were friable. They were then ground to a powder and incorporated in a basal ration consisting of casein, yeast, salt, agar, and dextrin or white corn, which constitutes a ration suitable for testing for the presence of vitamin A with rats. The dried tomato was mixed into these rations at a level of 2 and 5 per cent. To our surprise we found that even 2 per cent of dried tomato furnished enough vitamin A to allow young rats to grow in 16 weeks from a weight of 70 gms. to 290 gms. without any symptoms of ophthalmia or any other signs of vitamin A deficiency. Some of the females, as a matter of fact, produced a number of litters in the course of 18 weeks. This demonstrated to us an unexpected stability of vitamin A to the drying process, because the dried tomato, as a matter of fact, no longer resembled fresh material as to color; it had carmelized so that the final preparation was almost black.

It has been the object of our present experiments to determine how small an amount of tomato would suffice to furnish the rat with a sufficiency of vitamin A, and furthermore, to find out something about its distribution in this fruit. This problem has commercial as well as scientific significance, because there are now appearing on the market a number of tomato preparations for use as beverages. In some of these the entire tomato is macerated, freed from skins and seeds, and bottled as a permanent colloidal suspension of finely divided pulp. In other preparations, the tomato serum is separated from the pulp, leaving a pale yellow, perfectly clear solution. We have determined the distribution of vitamin A in a high grade commercial preparation.[1] Though sterilized by heat in the usual commercial manner, it contained sufficient vitamin C to cure a guinea pig of scurvy when given in amounts of 4 cc. daily. It was, therefore, evident that in its preparation it had not been unduly exposed to heat or oxygen.

Seventy-five white and piebald rats were used for our experiments. They were taken from stock at approximately 50 gms. in weight at 3 weeks of age and placed on screens in groups of 5 to 7. They were fed a basal synthetic ration free from vitamin A consisting of alcohol-extracted and heated casein 18, salt No. 40, 4, (37) agar 2, yeast 8, dextrin 68. The entire ration was treated with ultra-violet light from a Cooper Hewitt quartz mercury vapor lamp to supply it with vitamin D. Two hundred and eighty gram portions were spread out in a very thin layer in galvanized iron pans two feet square, and exposed for 30 minutes at a distance of 18 inches from

[1] The tomato used in these experiments was furnished by the Kemp Bros. Packing Co.

the burner. After 15 minutes exposure the material was stirred once. The lamp used was of a BY type which was run at 50 to 60 volts with a current density of 4 to 5 amperes.

The animals were fed as much of the ration as they cared to consume. No consumption records were kept. Symptoms of ophthalmia appeared at about the fourth or fifth week, but occasionally they appeared between the third and fourth weeks. With incidence of ophthalmia decline in weight usually resulted. When the ophthalmia gave every indication of being of a permanent rather than a transitory type, and when the eyes were severely erythemic but not purulent, the rats were taken for test. They were taken out of the different groups and segregated individually in small cages provided with screens. They were then given small amounts of the tomato preparation in addition to the basal ration. These were measured out by means of pipettes into small white glass dishes. The tomato was always consumed readily. The animals were observed daily and from week to week they were weighed and notes taken on the progress of their ophthalmic condition. In certain instances notes were taken daily, but this was the exception rather than the rule.

Some of the animals developed infections of the respiratory tract even before they were changed to the supplemented diet, and others developed this condition after the tomato feeding was started. Fortunately, these animals were few in number, and in most cases died shortly after the experiment was started so that the results were but little influenced by them. During the early course of the experiment, an attempt was made to distribute the members of the litters in such a way that they were apportioned equally among the different experimental lots. Some attention was also given to the equal distribution of the sexes; but later, when it was found that neither of these two factors was important, they were no longer given any consideration.

In order to conserve space the results of treating ophthalmia with tomato serum and whole tomato are presented in condensed form in Tables I and II and Chart 1. As seen in the tables most of the rats were started in what we designated progressive stage 3 of ophthalmia. This is marked by a persistent erythema, accompanied by swelling, sometimes with exudate and bareness of the lids, but with complete absence of purulency and the eyes either open or closed. This stage of ophthalmia is fairly definitely characterized and of a sufficient degree of severity to indicate slight improvement with treatment. On the other hand, when left untreated, it goes rapidly into the purulent stage, which we have designated as stage 4.

TABLE I

CURE OF OPHTHALMIA ON WHOLE TOMATO

Lot No.	Whole tomato daily cc.	Rat No.	Condition of ophthalmia when put on tomato	Condition of Ophthalmia Week by Week											
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
25	1/8	67	2	2	1	1	1	1	1	1	1	died			
		61	4	4	4	3	3	3	died						
		64	3	2	2	2	1	1	1	1	died				
		73	4	4	3	3	2	2	2	1	died				
18	1/4	31	3	4	3	2	2	2	2	2	1	1	cured		
		32	3	2	2	1	1	1	1	1	1	1	cured		
		33	3	3	2	2	2	2	1	1	1	1	cured		
		34	3	2	2	1	1	1	1	1	1	1	cured		
19	1/2	48	4	3	2	2	1	1	1	1	cured				
		50	3	2	2	2	2	1	1	cured					
		51	2	2	1	1	1	1	1	cured					
		52	3	2	2	2	1	1	1	cured					

Key to Ophthalmia Tables

4. Purulency accompanied by closed eyes with severe swelling and occasional blindness.
3. Absence of purulency, but persistence of erythema bareness, swelling and exudate with eyes open or closed.
2. Absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate eyelids bare.
1. Absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue.
0. Normalcy, absence of exudate, bareness of lids and scar tissue.

TABLE II

CURE OF OPHTHALMIA ON TOMATO SERUM

Lot No.	Tomato serum daily cc.	Rat No.	Condition of ophthalmia when put on tomato	Condition of ophthalmia week by week											
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
10	1	5	3	4	4	4	4	died							
		13	3	2	died										
		17	3	4	4	4	died								
		25	3	3	2	2	died								
11	2	9	2	2	1	1	1	1	1						
		1	3	4	4	4	4	4	died						
		18	3	3	3	2	2	1	1						
		28	3	3	2	2	2	2	2						
16	4	58	2	2	1	1	1	1	1	1	1	1	1	died	
		8	3	3	2	2	2	1	1	1	1	1	1	died	
		14	3	3	2	2	2	2	2	1	died				
		24	2	3	2	3	died								

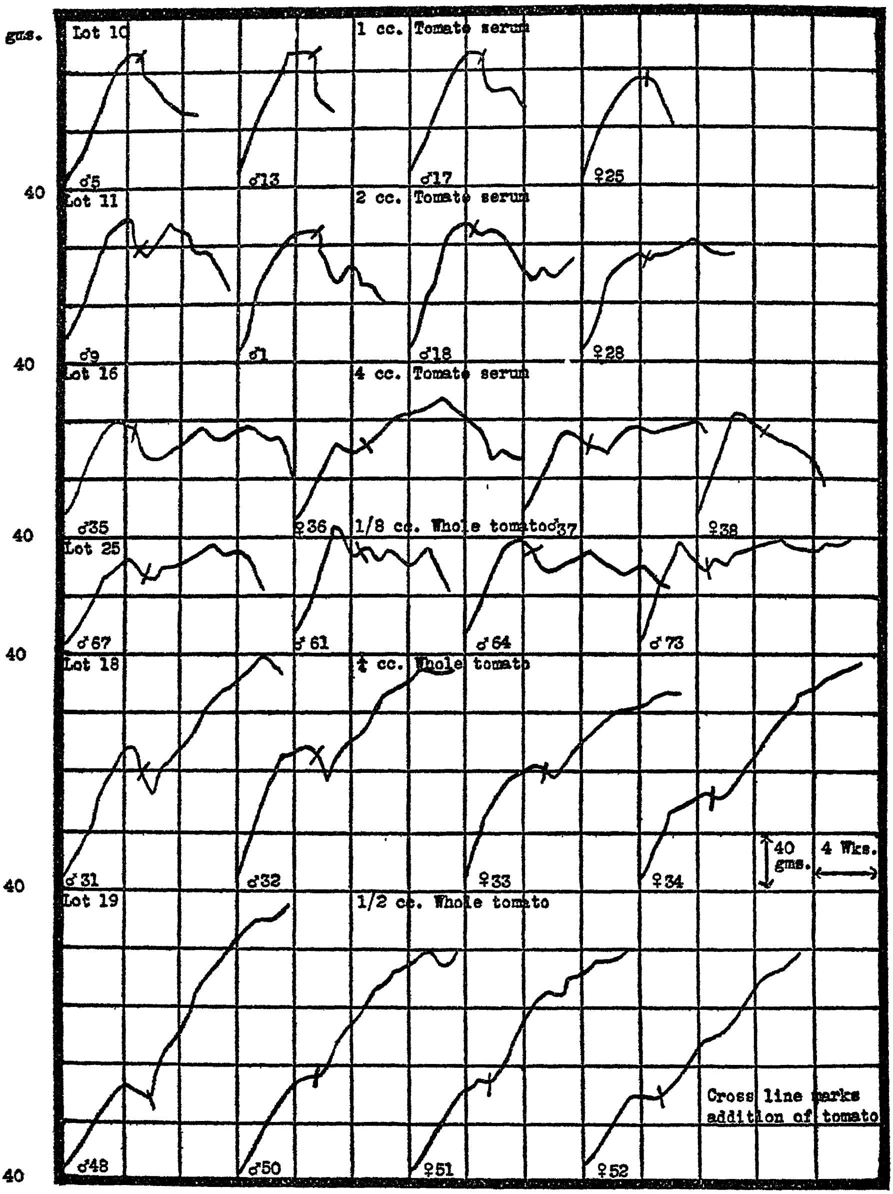

CHART. 1. GROWTH PROMOTING PROPERTIES OF WHOLE TOMATO *vs* TOMATO SERUM

Table I shows recovery from ophthalmia with the whole tomato preparation on 1/8 of a cc. daily at a very slow rate, but the amount of vitamin A contained in this daily supplement was not sufficient to maintain normal health. By the end of the ninth week all of the animals in this group had died. On 1/4 cc. tomato daily, the ophthalmia had been completely cured by the tenth week, and on 1/2 cc. the same result had been obtained by the eighth week and almost by the seventh. This proves beyond question the remarkable potency of tomato preparations in vitamin A, especially when it is remembered that this material contains only 4.44 per cent solids—which means that 1/4 cc. daily represented only approximately .16 per cent of the total daily intake of dry matter, or 11 mgs. daily.

Table II shows the results obtained with so-called tomato serum. It will be noted that on 1 cc. of this preparation daily, the rats had all died by the fifth week. On 2 cc. one animal died during the sixth week, and the others showed only a slight retrogression in the severity of their symptoms. On 4 cc. one animal died during the fourth week and the others improved very slowly, but ultimately died in the eleventh. Evidently other organs than the eyes were affected critically by the deficiency of vitamin A.

Comparing the results obtained on whole tomato with those on tomato serum, it is noted that the response with 4 cc. of the serum approximates fairly closely the response obtained on 1/8 cc. whole tomato. From this standpoint it may be concluded that the whole tomato contained about 32 times as much vitamin as the serum. Growth responses obtained by feeding the tomato preparations correlate very well with the ophthalmia indications. This is shown in Chart 1, which presents graphically the growth responses of the same animals of which the ophthalmic records are presented in Tables I and II. Maintenance on 1/8 cc. of whole tomato approximates very closely that observed on 4 cc. of tomato serum and 1/2 cc. of whole tomato led to approximately normal growth performance. Both growth and ophthalmic reactions show that in whole tomato there is approximately 32 times as much vitamin A as in the clear tomato serum.

The comparatively recent work of v. Euler and co-workers (38) found .005 mg. carotin per rat daily, effective as a source of vitamin A. Moore (26) confirmed the potency of carotin but Duliere, Morton, and Drummond (25) found no activity in a specially purified carotin. Lycopin, the red pigment of tomato, has been found inactive (39). We are quite certain as to the inactivity of lycopin because Mr. Baumann, in this laboratory, has found it inactive in dosages of .07 mg. daily in repeated experiments. With carotin purified by 20 precipations with methyl alcohol from chloro-

form solution in large volume, .007 mg. daily was found very active. However, the experience of various workers in connection with vitamin D naturally makes one rather skeptical as to whether the ultimate state of separation of a preparation into its physiologically active units has been attained not excluding the complicating possibility that a number of substances may elicit the same physiological reaction. We have found an abundance of yellow pigment extractable from tomato pulp with petroleum ether; the serum, however, contained but little.

Vitamin D

So far no mention has been made of vitamin D. As this is one of the vitamins upon which attention is widely focused, we obtained some data on its presence when we used tomato as a source of vitamin A in experiments which compared different cereals for their rickets-producing properties. We found that rats receiving additions of 2 cc. of tomato daily showed no increase in bone calcification. As it is difficult to give large quantities of tomato to rats, because they refuse to consume it, we later evaporated various amounts of tomato on our basal ration 2965 (40). This was done by the use of an air current at a temperature not exceeding 60°C. Ten, 20, and 40 cc. of tomato were evaporated on each 7 gms. of ration. These rations were prepared in quantity and then stored. They were fed in a series, together with ration 2965 as a control, to litter mates taken from four litters of rats weighing from 52 to 60 gms. This consumption was equalized so that by the end of the fifth week all the rats had consumed the same amount of ration. They were then killed and the fat-free femurs analyzed for ash.

The results are presented in Table III. They reveal an increase in weight

TABLE III
CALCIFYING ACTION OF TOMATO

Rat Nos.	Tomato Evaporated on 7 gms. ration	Food consumed	Tomato solids	Femurs weight	Femurs ash	Femurs ash
	cc.	gms.	gms.	mgs.	mgs.	per cent
65–68	0	1063	0	0.109	0.044	40.4
73–76	10	1048	66	0.127	0.055	42.8
77–80	20	1043	132	0.142	0.071	49.8
81–84	40	1064	269	0.164	0.089	53.6

of femurs, femur ash, and percentage of ash with increase in the amount of tomato in the ration. It is evident that with the consumption of large amounts of tomato, calcification of bone was improved and growth was

likewise improved. Without tomato the final weight of the animals was from 80 to 85 gms.; with tomato the animals weighed from 90 to 107 gms. It should, however, be mentioned that with such a large proportion of the solids consumed composed of tomato solids, which in one case amounted to 25 per cent of the ration, the calcium carbonate intake was reduced and the calcifying power of the ration was correspondingly improved. Taking this into consideration it is evident that the calcifying power of tomato actually is very low.

As tomato is rich in both vitamins A and C and therefore constitutes a valuable supplement for the diets of infants and children, it appears that it would be desirable in many instances to have it likewise potent in vitamin D. As the production and addition of vitamin D in the form of an irradiated ergosterol solution offers no special difficulties, we have carried out some experiments to determine if irradiated ergosterol would maintain its activity in such an acid medium as tomato represents. A determination of the pH of our tomato preparation showed it to have a value of 5.77.

Ergosterol prepared from yeast[2] was activated by exposure, in ether solution, to the radiations of a quartz mercury vapor lamp. After evaporation of the ether in vacuo it was brought into solution in alcohol and added to the tomato before it was bottled and sterilized in the usual commercial manner. One hundred fifty cc. of alcohol carrying 5 mgs. ergosterol were added to 15 quarts of tomato. This amount was much larger than necessary for the preparation of a commercial product of the desired potency, but it was added in excess in order that we could readily determine the percentage of destruction which might result on storage with the small amounts of tomato which rats might be induced to consume. We reserved part of the ergosterol for standardization, and other portions were added to tomato and then tested immediately before and after sterilization at 15 pounds pressure for one hour.

The bulk of the fortified tomato was placed in storage at room temperature in an incubator run at 37° and in a refrigerator. The potency of these was again determined after 2 and 13 months storage, using as controls some of the same tomato which had been freshly fortified with irradiated ergosterol, both sterilized and unsterilized.

The tests for potency were made by means of the Johns Hopkins line test technique (41) using rats of approximately 60 gms. in weight and 3 to 4 weeks of age. These were made rachitic by feeding for 3 to 4 weeks on

[2] The ergosterol was kindly furnished by The Fleischmann Co.

ration 2965. The tomato was then fed daily for 10 days in small dishes. At the end of the ten-day period the distal ends of the radii and ulnae were examined for calcium deposits.

The results after 13 months are presented in Table IV. Earlier results are not presented because they were identical with those given. All of them showed no loss of potency after storage even at 37° in an incubator.

TABLE IV

CALCIFICATION ON 0.1 CC. TOMATO PLUS ERGOSTEROL DAILY

Ergosterol	Litter No.	Rat No.	Initial weight gms.	Final wt. gms.	Average consumption R. 2965 gms.	Line Test
Freshly added	88	8938	88	105	11.0	+++
	89	8942	78	87	7.5	++
	90	9012	105	113	7.5	++
	91	9002[1]	96	106	6.7	++
	91	9006[2]	83	92	5.7	++
	96	9295	83	87	6.7	+
	96	9296	95	102	8.0	+
	4786	499	87	97	8.2	++
	4787	505	97	103	5.7	++
Freshly added and sterilized	90	9010	112	114	6.5	++
	91	9005[2]	88	93	6.6	++
Stored in refrigerator 13 months	4784	488	87	94	9.2	++
	4785	493	88	91	6.6	++
Stored at room temrature 13 months	4782	477	88	92	9.6	++
	4783	483	78	85	8.1	++
Stored in incubator 13 months	4780	466	89	79	6.4	++
	4781	671	89	100	7.0	++

SUMMARY

The literature on the vitamin content of tomato has been reviewed. Experiments designed to determine the distribution of vitamin A in red tomatoes showed that freed from skin and seeds the pulp contains approximately 32 times as much vitamin A as the clear yellow serum. From the nutritive standpoint, therefore, there is no justification for marketing a filtered tomato juice in preference to a juice containing the pulp in suspension. Vitamin D added in the form of irradiated ergosterol was found

to have maintained its activity after sterilization, followed by 13 months storage at 37° C.

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Editorial Review

THE FUEL OF MUSCULAR ACTIVITY OF MAN

THE subject of the source and kind of material which furnishes the energy for the performance of muscular work is one that has engaged the activities of many physiologists and students of metabolism. From time to time new studies appear which are either repetition of previous work by later and more accurate methods, or are actuated by new points of view which arise from new findings in physiology. The subject seemingly at times appears to be completely settled to the satisfaction of many physiologists, then a new investigation occurs with results which are not in line with either previous findings or with current theories. Thus a new impetus to investigation is given, and a renewed activity takes place in a field which has previously seemed to have been exhausted.

Chauveau (1896) made an observation upon Tissot of the respiratory quotient at intervals during 70 minutes going up and down stairs together with those of the rest periods preceding and following the period of activity. As the respiratory quotient rose from 0.74 to 0.75 at rest to 0.95 during work, Chauveau concluded that muscular activity was accomplished at the expense of carbohydrate, and that carbohydrate was formed from fat. Subsequently (1897, 1898) he made studies on a dog with diets of predominantly carbohydrate or fat and from studies of body weight changes, he concluded that fat was of nutritive value for muscular activity only to the extent that it could be converted into glycogen. Chauveau's researches have been criticized by Zuntz (1896, 1898) who, together with his associates, made a large number of studies upon the metabolism of muscular work with especial reference to the source of energy, and came to the conclusion (Zuntz, 1911) that during work the nutrients catabolized were essentially the same as during rest, and also that the oxygen absorbed per unit of work was essentially the same whether carbohydrate or fat was burned.

Benedict and Cathcart (1913) found that the respiratory quotient during work was influenced by the character of the preceding diet as the average quotient during work after a carbohydrate-rich diet was 0.90 and after a carbohydrate-poor diet was 0.82. They also found that, in general, the more intense the work, the higher was the respiratory quotient. They came to the conclusion that the values indicated that during the period of severe

muscular activity the character of the catabolism is changed by an increase in the carbohydrates burned, with a draft upon the carbohydrates of the body for such a purpose. At the completion of muscular work, the specific metabolism due to muscular work ceased, and the picture was one of a body subsisting upon a depleted storage of carbohydrate as shown by the lower respiratory quotient after muscular work.

Krogh and Lindhard (1920) made a very extensive study on the relative value of fat and carbohydrate as sources of muscular energy. The work was performed on a Krogh ergometer. The respiratory exchange was measured in an open circuit chamber apparatus, the outcoming air of which was analyzed by a gas analysis apparatus with an accuracy of 0.001 per cent. The subject usually rode the ergometer about two hours, and the measurements were made in three periods of 20 to 30 minutes after the subject had been riding for at least 20 minutes. The oxygen absorption varied between 860 and 1434 cc. per minute. In the three best series of experiments, the energy per unit of work varied from 4.6 calories at a respiratory quotient of 0.71 to about 4.1 calories at a respiratory quotient of 1.00. Their experiments led them to the following hypothesis: The proportion of carbohydrate to fat catabolized depends upon the relative available quantities of the two substances and is substantially the same during rest and during work. When the available supply of carbohydrate is in excess of that of fat, fat is formed from carbohydrate. This formation becomes distinct at quotients above 0.9. Sugar or allied substances are formed from fat (and protein) when the available supply of fat is in excess of carbohydrate. This formation becomes distinct below 0.8.

H. M. Smith (1922) determined by means of a closed circuit apparatus the respiratory exchange of men during grade walking. The duration of the periods was about 12 minutes and usually several periods were run in succession. The values of the respiratory quotient tended to increase as the grade and speed increased. The majority of the respiratory quotients up to approximately 600 kilogrammeters of work per minute were within the limits of 0.80 to 0.87 and for 700 to 1000 kilogrammeters of work per minute almost half of the respiratory quotients were 0.90 or above and none was below 0.85. For more than 1000 kilogrammeters per minute the respiratory quotients grouped around 0.93 and none was below 0.90. Two determinations with work greater than 1300 kilogrammeters per minute gave respiratory quotients of 0.97.

The author stated that short periods such as these with 600 kilogrammeters or less of work per minute did not tend to alter the character of the

respiratory quotient, but with moderately heavy or heavy work involving over 600 kilogrammeters per minute the body altered its metabolism by a tendency to a selective consumption of its carbohydrate reserve. These experiments definitely show a relationship between the character of the fuel for muscular activity and the intensity of the activity. In other words, the more intense the activity the higher was the respiratory quotient.

Furusawa, Hill, Long, and Lupton (1924) published a series of experiments on muscular exercise and the oxygen requirement. The study was principally to determine the oxygen requirement as distinguished from the oxygen absorption for different amounts of activity of very short duration consisting of walking, flat running, and standing running. In a group of 13 experiments in which the duration of the exercise varied from 16.3 to 18.3 seconds and the total duration of exercise and recovery varied from 6.2 to 10.6 minutes, there was a considerable variation in the total respiratory quotients and the respiratory quotients of the excess metabolism. The authors state that they were made before they realized the considerable duration of the recovery process after severe exercise. In several of them they say that the recovery was not quite complete and the oxygen requirement as determined was appreciably too small.

In two succeeding tables they give 11 experiments on K. F. with the particular object of determining the oxygen requirement. In the discussion of the results of these experiments they point out that the excess quotient, *i.e.*, the quotient of the excess carbon dioxide and the excess oxygen over the base line due to work and recovery, varied from 0.93 to 1.13 with an average of 1.02 in one group and 1.04 in the second. They state that the interval allowed for recovery was admittedly not long enough for complete recovery.

The authors concluded that with man undergoing prolonged exertion the respiratory quotient certainly is not unity. "It would seem possible, however, that in man an element of muscular exercise, of such short duration that it does not seriously affect the general metabolism of the body, may be paid for primarily by the oxidation of carbohydrate and that if the effort be prolonged the carbohydrate oxidized must be restored at the expense of other substances." They were inclined rather to regard the high respiratory quotient of the recovery process of these short-lived efforts as a genuine effect. They stated, however, that the conclusion was so important that more careful work was needed with longer recovery periods and that at that time the conclusion must be regarded as tentative. These experiments were made later by Furusawa.

Furusawa (1925) made the most striking observations upon the character of the metabolism due to short periods of muscular work. The exercise of standing-running was employed. The subject rested for 20 to 30 minutes, during which period the author states that the recovery process from previous muscular activity should be completed. After a 10-minute sample of expired air was collected which gave the first base line value, the exercise was taken and the expired air from the beginning of exercise to recovery was collected in a large Douglas bag. Another 10-minute collection of expired air was taken after recovery which gave the second base line value. These two values gave the basis from which the excess metabolism was calculated.

The first group of Furusawa's investigation contained 13 experiments on two subjects with periods of exercise from 0.5 to 1.0 minutes, with a total time of collection of 10 to 40 minutes. The basal respiratory quotients varied from 0.81 to 0.93. The respiratory quotient of the excess metabolism was close, with few exceptions, to unity. The extra work involved, however, was small so that the maximum amount of carbohydrate catabolized during the period of 40 minutes, including that due to basal metabolism, was 10.7 grams and of this 6.3 grams was due to the excess metabolism. The minimum total carbohydrate catabolized in the work and recovery periods was 1.7 grams and the minimum excess metabolism of carbohydrate due to exercise was 0.4 gram. The oxygen requirement for the work varied from 700 to 9000 cc. per minute. The second group consisted of 4 experiments with long-continued exercise from 10 to 30 minutes and the time of collection varied from 55 to 70 minutes. The total carbohydrate used in these cases varied from 17 to 35 grams and the carbohydrate in the excess metabolism due to exercise from 9.8 to 23.4 grams. The greatest deviation from unity in the respiratory quotient of the excess was 0.04. The average oxygen intake was of the order of 700 cc. per minute. The author stated that in the 30-minute exercise 140 grams of carbohydrate must have been oxidized. (This is evidently an error; presumably 140 calories was intended.)

The next group contained several experiments at higher speed with an oxygen requirement of 1.9 liters per minute. Here again the greatest deviation from unity was 0.06 for the excess metabolism and the total carbohydrate metabolism varied from 39 to 56 grams in exercise and recovery, whereas the excess metabolism of carbohydrate varied from 29.9 to 49.5. The respiratory quotient of the excess metabolism in the 28- and 30-minute experiments was 0.94 and 0.88. According to the author, only following the

oxidation of 300 grams of carbohydrate could the oxidation of fat be detected. (This is obviously an error.)

A group of 12 experiments was performed in which the diet preceding the experiments was mainly fat. The basal quotient varied from 0.65 to 0.77. The time of exercise was from 0.33 for 272 steps per minute to 2.0 minutes at 146 steps per minute. The time of collection varied from 20 to 35 minutes. The carbohydrate in the total metabolism of exercise and recovery in this group was from 1.4 to 8.2 grams, whereas the carbohydrate of the excess metabolism due to exercise was from 1.1 to 6.3. In spite of the low initial quotient the respiratory quotient of the excess varied from 0.98 to 1.06. The single experiment in which the base line quotient of 0.65 was found is given in somewhat more detail and it is shown that the respiratory quotient both before and after the exercise as well as the carbon dioxide and oxygen were nearly identical.

Furusawa concluded that the metabolism due to a short element of exercise was the same, namely, the oxidation of carbohydrate only, whether a great amount of carbohydrate or only fat is supplied to the body. This suggested that the position of carbohydrate in muscular work was unique, and that fat was used only after conversion into carbohydrate.

A fifth group of experiments after a high fat diet at 146 steps per minute with a basal quotient from 0.72 to 0.76 and a duration of exercise from 4 to 9 minutes gave as a respiratory quotient of the excess from 0.92 to 0.97. In this group the carbohydrate metabolized in the total period of exercise and recovery varied from 12.9 to 23.0 grams and the excess metabolism varied from 11.5 to 19.5 grams.

The author concluded that with short-lived muscular exercise even when the basal respiratory quotient reached 0.71, the exercise was performed at the expense only of carbohydrate. From these facts it could be concluded that with exercise of short duration in which no change in the general metabolism of the body as a whole might be expected, the human body acted as though it were an isolated muscle in which carbohydrate only was oxidized. The primary fuel of contraction, therefore, in the human muscle is carbohydrate, and fat or protein is presumably used to replenish the carbohydrate store which has disappeared.

From a general survey of Furusawa's experiments it would appear to the reviewer that when the diet was normal, there was enough available reserve of carbohydrate to draw upon for these short periods of exercise, so that there was no need to call upon fat even in the long-continued experiments of 30 minutes' duration where a total of 35 grams of carbohydrate

were used in a period of 70 minutes. It was only when the draft upon carbohydrate was about 40 grams, that the call upon fat was shown. In contrast to the experiments with normal diet are the experiments with fat diet in which small quantities of carbohydrate for the most part were catabolized during the period of work and recovery or due to the work itself, but here the draft upon fat was at a lower level of carbohydrate catabolized than after normal diet as it began at apparently 12 to 13 grams of total carbohydrate catabolized. In the opinion of the reviewer, the results in the experiments of Furusawa were due to the small amount of work involved and to the fact that even on a fat diet there was still enough carbohydrate available to supply the energy for exercise and recovery.

Hetzel and Long (1925) determined the respiratory exchange of three diabetic patients before, during, and after muscular exercise. They used the same methods that were used by Hill and co-workers. The observations were divided into three groups depending upon the time of the last injection of insulin: 1—insulin injection within the last 6 hours; 2—without insulin for periods from 10 to 17 hours; 3—without insulin from 20 to 27 hours. The exercise was that of "standing-running." The preliminary period varied from 20 to 60 minutes during which the subject rested, then a collection for the resting sample of from 10 to 15 minutes was made. At the end of the recovery another resting sample was collected from 10 to 15 minutes and, if the initial and final resting metabolisms did not agree within reasonable limits, the experiment was discarded. The time of recovery was from 30 minutes for the half-minute periods of exercise to two hours for the 8-minute periods.

In the exercise of short duration with insulin taken within the last 6 hours or within the last 10 to 17 hours, the average of the respiratory quotient of the excess metabolism in 17 experiments was 0.99 as compared with a previous average respiratory quotient of 0.76. In a second group in which the exercise was of longer duration with a recent administration of insulin, the average respiratory quotient of the excess metabolism was 0.85 as compared with a resting quotient of 0.76. The authors calculated that 15 per cent of the energy at rest was derived from carbohydrate, whereas during work 50 per cent was derived from carbohydrate.

In the third group with exercise of short, moderate, or long duration without recent administration of insulin, the average respiratory quotient of the excess metabolism was 0.80 as compared with a previous resting value of 0.76.

The authors concluded that in the absence of insulin the respiratory

quotient of the excess metabolism apparently is never unity, however short the interval of exercise, and that the results of diabetic patients without insulin represent an exaggeration and extension of the results found with normal men on a fatty diet. They believe that the carbohydrate is restored by the transformation of some other substance before the recovery is complete, that instead of the muscles being able to use fat directly, it is simpler to suppose that they can use carbohydrate only and that insulin is necessary only in order to maintain the carbohydrate at sufficiently high levels for use in oxidations in recovery. Rapport and Ralli (1928) stated that they were unable to agree to the above interpretation of these results, because, if true, one would expect increasingly lower quotients of the excess metabolism as the exercise was prolonged, but their analysis of Hetzel and Long's results did not show such decreases in the quotient.

Henderson and Haggard (1925) studied the respiratory exchange of a group of Yale rowers during and subsequent to a period of exercise on a rowing machine. The expired air was collected in a spirometer of 400 liters capacity and two large Douglas bags and the analyses were made by a Henderson-Orsat apparatus. The subject removed his outer clothing and, after adjusting the foot board, sat quietly on the sliding seat of the rowing machine for 10 minutes. After this partial rest, his expired air was collected for five minutes. He then rowed at a uniform rate for 5 minutes. The whole expired air for this period was collected. Then he sat still for fifteen minutes during which the expired air was collected in a Douglas bag for two minutes, then three minutes and then ten minutes.

The authors concluded that the values found showed that whatever proportion of fat and sugar the subject burned during rest prior to work, he continued to consume during a short period of great exertion. Sugar was therefore not the sole fuel of muscular energy.

The respiratory quotients at rest before work were extremely variable in this group of subjects, from two at 0.66 to 0.67, which the authors questioned, to one at 0.94, including one each at 0.75 and 0.74. In the great majority of cases the respiratory quotient during five minutes of work was higher than the quotient of the rest periods. Similarly, this was true with regard to the two minutes of recovery and the next three minutes of recovery. The respiratory quotient of recovery and work was higher than the rest quotient in 8 out of 12 experiments and the respiratory quotient of the excess varied from 0.72 to 0.96, and was higher than the rest quotient in 8 out of 12 cases. The total carbohydrate used in these experiments in work and recovery varied from 2.2 grams with one subject, whose basal

quotient was 0.73, to 24.1 grams. These are not, therefore, extraordinarily high values. If one of the conditions for securing a respiratory quotient of unity for the excess metabolism due to work is that the draft upon carbohydrates shall be small, we would expect a respiratory quotient of unity in this group. However, in the opinion of the reviewer there were several factors in this investigation which would hardly conform to the conditions which are optimal for obtaining a respiratory quotient of unity due to work. The conditions for obtaining the pre-work quotients were not ideal. After a ten-minute rest one could hardly expect to obtain the real base line quotient particularly after the muscular activity involved in undressing and adjusting the apparatus. In addition, the recovery periods were not continued long enough to approach the values of the rest period. The last oxygen value was frequently over 100 cc. higher than the pre-work value, and, therefore, the subjects had not recovered and the experiments did not conform to the requirement. The minimum difference between the pre-work value and the last recovery value is 50 cc. The experiments of Henderson and Haggard indicated that in the majority there was an increase in the proportion of carbohydrate utilized in the period of exercise and recovery.

Wilson, Levine, Rivkin, and Berliner (1927) studied the effect of moderate exercise upon the respiratory quotient with four children in which a closed circuit chamber respiration apparatus was used. In one group, the rest and exercise periods were each of two hours' duration and were made on the same day on two of the subjects and on different days with the other two. In the experiments with exercise the precaution was taken to have the exercise stopped before the end of the period in order to allow conditions in the chamber to reach a steady state. This period was from 30 to 25 minutes before the end. The respiratory quotients during the rest experiments and the experiments with exercise were nearly identical. The percentage increase due to the activity varied from 17 to 52. The respiratory quotient of the extra metabolism varied from 0.71 to 0.87 and in nearly all cases was very close to the respiratory quotient of the rest experiments. The authors concluded that the results appeared to furnish further evidence in favor of Lusk (1925) and his co-workers that fat can be burned directly under stress.

The reviewer is of the opinion that a closed circuit chamber respiration apparatus is not suitable for measuring the change in the respiratory quotient due to such small increases in metabolism, especially when the rest and exercise experiments take place on different days.

Marsh (1928) made an extensive study of the fuel of muscular activity during work and recovery. Five boys were used as well as four adult laboratory workers and seven medical students. With one of two boy subjects used extensively no attempt was made to control the diet or activity. The other boy ate a standard breakfast and lunch on the experimental day. The respiratory exchange was measured by means of the Benedict Universal apparatus. The work was performed on bicycle ergometers. The author states it is quite as necessary to secure recovery of the original rate of carbon dioxide elimination as to secure recovery of the original rate of oxygen absorption and that results would not be accurate if the recovery was not complete, or a diminished respiratory exchange followed immediately upon complete recovery.

The subject rested 20 minutes to one half hour. He then took his position upon the bicycle, adjusted the mouthpiece and noseclip and, after the breathing had become regular and normal, the first resting period of 7 to 10 minutes was made in this position. After the rest period, the subject was disconnected and sat in a chair while the absorbers were being weighed and then followed the work and recovery periods with no interruption between them.

The respiratory quotient of the excess was obtained by deducting from the carbon dioxide and oxygen of the work and recovery period the values of the first rest period. It was found that no material difference was made in the average values whether the first rest period or both rest periods were used in the calculation. The author points out that there may be overventilation at the beginning of work but, if the recovery period is sufficiently long, a period of retention of carbon dioxide would in time balance the blowing off and thus restore the equilibrium. It is essential that the recovery be continued to this point as well as to the point where the rate of oxygen absorption equals that preceding the work period.

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The author made a special series of experiments to determine the length of time for recovery and she found that it was possible to continue too long, because it led to an irregularity which might be explained by the unrest caused by sitting on the ergometer. She came to the conclusion that a period of only 8 minutes was sufficient for the work which was done by this subject, a boy. A series of 37 experiments was made with one boy in which the average basal quotient was 0.87 and the average quotient of the excess metabolism was 0.78. In another group of 11 experiments with the same subject, the average basal quotient was 0.91 and the excess quotient was 0.88 and in general on a much higher level than the earlier series with

the same subject. The results in the latter group are ascribed to the subject's better physical condition.

With one adult subject three types of experiments were made, one with a normal diet, another with a high carbohydrate diet, and a third with a high fat diet. The first group consisted of 16 experiments on a normal diet. Half the experiments were of 5 minutes' duration of work with 8 to 12 minutes for recovery and the other half 5 to 10 minutes work with 11 to 17 minutes for recovery. The work ranged from 4.48 to 13.7 calories. The average respiratory quotient of the excess metabolism was 0.95, varying from 0.88 to 1.05, and the average basal quotient was 0.82 with a range from 0.75 to 0.86. Part of the experiments were in the post-absorptive condition and part from 3 to 5 hours after breakfast. This latter condition did not seem to make any positive difference in the pre-work respiratory quotient or in the respiratory quotient of the excess metabolism. The author states that the high respiratory quotient in the work and recovery may be associated with the subject's physical condition and perhaps state of maturity as compared with the boys'.

In the first group with the carbohydrate diet the average respiratory quotient before work was 0.83 and the average respiratory quotient of the excess was 0.96 with a range from 0.88 to 1.05.

In the first group on a fat diet, the base line quotients averaged 0.73 with a range from 0.70 to 0.80, thus showing definitely the effect of the diet upon the quotient. The excess quotient, however, was 0.83 with a range from 0.79 to 0.86. Thus in spite of the fat diet, there was some carbohydrate burned during muscular work. The recovery was apparently slower on the fat diet both as regards carbon dioxide and oxygen even when the period was continued as long as 28 minutes.

A carbohydrate series followed with results essentially similar to the first series. The average respiratory quotient of the excess metabolism in 10 experiments was 1.00 and the quotient before work was 0.88 and 0.91 in the two groups of this series. This was succeeded by another series on the high fat diet. The average basal quotient was 0.74 ranging from 0.71 to 0.80. The quotient of the excess metabolism was 0.80 and the average quotient after the work ceased was 0.81. This seems singular because as the muscular work caused a greater proportion of carbohydrates to be metabolized than during rest, one would expect that the quotient after the work ceased would be as low if not lower than the quotient preceding the work. This may have been due, as the author points out, to the fact that the recovery of the carbon dioxide to resting value was not obtained.

The author concludes that the response to the work varied with the individual and that there does not seem to be a uniform response to muscular work in so far as the change in respiratory quotient is concerned.

This study is of interest to the reviewer primarily in connection with the study on the adult in which a long series of experiments was made. The conditions for obtaining changes in the respiratory quotient due to the effect of small amounts of work were not ideal, in the first place because the base line period was obtained immediately after the subject had changed his position, in the second place because the recovery period was obtained in a condition which ultimately led to discomfort and in some cases to a shifting set of recovery values, as pointed out by the author.

The group of experiments on the adult is consistent in that both carbohydrate groups gave high respiratory quotients in the excess metabolism as well as with the normal diet and the experiments with the fat diet pointed in the same direction although not so positively as the carbohydrate diet. In other words there was a definite increase in the carbohydrate metabolized due to muscular work even after a fat diet which resulted in a very low base line quotient.

Lindhard (1928) sought to test the general thesis of A. V. Hill and co-workers that a short period of muscular work proceeded exclusively at the cost of carbohydrates. He criticized the series of experiments on the material upon which Hill and his co-workers (Furusawa, Hill, Long, and Lupton, 1924) drew their conclusions. He particularly called attention to the variations in respiratory quotient of the work ranging from 0.93 to 1.13 with an average of 1.03. He referred to other tables in the same article that had a wide variation in the respiratory quotient of the excess metabolism due to work and that the authors do not mention these experiments. He illustrates the fact that with a very moderate amount of work the respiratory quotient of the excess metabolism is not unity but below unity. However, in this connection it must be mentioned that the authors themselves stated that those experiments were carried out before the necessity for a longer recovery period was recognized and even in the experiments from which the average quotient of 1.03 is drawn, they stated that the period of recovery was too short, that the quotients were likely to be too high, but that the evidence pointed in the direction of carbohydrate being a source of muscular work when very short periods of work are performed and that the idea was wholly tentative with experiments planned in the future. Lindhard also criticized as worthless the work of Simonson (1926) in which a range of quotients for excess work from 0.71 to 1.31 was found

with an average of 0.99. Simonson's studies were made with the Zuntz-Geppert apparatus. It is a pity that this serious and conscientious worker should have published so much material based upon a technique wholly inacceptable to research workers in this field.

Lindhard's experiments were performed with a Krogh ergometer with 60 revolutions per minute and a range from 892 to 1115 kgm. per minute. The expired air was collected in Douglas bags suspended on a wire net above the subject. The subject came to the laboratory in a post-absorptive condition and sat immediately and began shortly to breathe through a mouthpiece and valves, filling the bags which were to be used for the experiment. After one-half to three-quarters of an hour the subject sat on the ergometer and after another 15 to 20 minutes, he applied the nose clip and mouthpiece and breathed to the atmosphere for 20 minutes before beginning the rest experiment which was six to seven minutes' duration. Several minutes after the rest experiment, the work experiment began. Three bags were used for continuous collection of expired air during the work period and recovery. If more than three were necessary there was a pause between the last and the fourth.

Sample experiments are shown graphically in which the course of the oxygen absorption and carbon dioxide are given by periods during work and recovery. He then discusses the advantages and disadvantages of the method and the fact that because of the limit as to the length of time during which the subject could remain upon the ergometer during the recovery period, breathe through the mouthpiece and still remain comfortable, the recovery period was of necessity shortened.

Lindhard's method of application of the periods of recovery was different from that of Hill because he included only the periods of recovery to the point where the respiratory quotient fell to the respiratory quotient of the pre-work period without reference to the fact that the respiratory quotient may rise to a higher level after this point is reached. In other words, during the recovery he includes only those periods up to the point where the line showing the course of the respiratory quotient first cuts the value of the base line.

He had 12 experiments with two subjects in which the basal respiratory quotients before work varied from 0.71 to 0.88 and he states that particularly with one subject in which the range was the same, these variations were real, due to the training and to the variation in diet. A quotient of 0.71 seems to the reviewer unusually low for a subject on an ordinary mixed diet and we would look with doubt upon the quotient below 0.76 un-

less the subject had been without food for more than 12 hours and had had a low carbohydrate diet. The range of the respiratory quotients in the rest period after the work varied from 0.74 to 0.89 and in most cases was slightly higher than the respiratory quotient before the work. His comment regarding this is that it was only apparently the case because these numbers refer to the middle of the period and if one takes the end of the line where the curve of the quotient cuts the rest level one will see that the real quotient at the end of the rest period corresponds to that before the work.

The total excess metabolism varied from an oxygen consumption of 1938 cc. in 12.79 minutes to 64.7 liters in 50.5 minutes. There was thus an extremely wide variation in the extra metabolism. Similarly the work period varied from 1.93 to 36.28 minutes. The recovery periods were short, from 10.62 to 24.32 minutes. The respiratory quotient of the excess metabolism varied from 0.82 to 1.205 and the majority of the quotients are above 0.90. There was a wide range in the total carbohydrates catabolized during the work period and recovery period. The variation was from 1.6 in 14.6 minutes to 59.2 grams in 51.2 minutes. Lindhard stated that these were typical experiments.

There is a uniformity neither in the basal respiratory quotients nor in the respiratory quotients at work, nor is there any parallelism in the gradual changes. In three of the experiments, the second rest value had an oxygen absorption from 35 to 65 cc. per minute higher than the first resting periods. With conditions so variable and with results also so variable, in the opinion of the reviewer it is questionable whether these experiments are of significance in solving the question of the source of energy for muscular activity of short duration.

Bock, Vancaulaert, Dill, Fölling, and Hurxthal (1928) made a study of the respiratory quotient during work, particularly with reference to the steady state. According to them, the steady state may be associated with an oxygen debt provided it is not cumulative, but is principally acquired in the period at the beginning of work. A steady state cannot be maintained by the average man not in training for a continuous period of work on a stationary bicycle when the oxygen requirement of the work done exceeds about 2 liters per minute. Some of their recent experiments running on a treadmill indicate that an oxygen intake of 2.5 liters per minute can be continued for 2 hours with this type of exercise.

Two experiments were made with the subject D. B. D. The metabolic rate was nine times that of the resting state. The lactic acid and total

carbonic acid of blood returned to a resting level at the end of 60 minutes of work. The average respiratory quotient for the total metabolism was 0.96 and for the excess metabolism was 0.97. Another experiment on the same subject in the post-absorptive condition showed that a steady state was reached at the end of 4 minutes and at the end of work nearly basal conditions were obtained in fifteen minutes. Most of the excess oxygen taken in during this period was due to the oxygen debt acquired in the first three minutes of work. A small part of it might be referred to the gradual return of the machine to the state existing before the work was begun. The average respiratory quotient of the total metabolism was 0.93 and of the excess metabolism in the working period was 0.97.

Tables are given showing the respiratory quotients for the total metabolism from the beginning of work until the end. With D. B. D. the R. Q. began at 0.98 and fell to 0.88 in the 50 to 59th minute. With A. V. B. the fall in 44 minutes was from 1.02 to 0.88.

The authors adhere to the following hypothesis with regard to the significance of the respiratory quotient as a means of interpreting the processes of catabolism. The primary source of energy for muscular contraction in earlier stages of work is carbohydrate. During moderate work glycogen is utilized, but at the same time the velocity of reactions involved in its synthesis from fat or other processes by which other sources of energy may be utilized nearly keep pace with the rate of glycogen utilized. The resultant respiratory quotient will rise only slightly above the resting level. With more severe work the muscles utilize stored glycogen with a velocity of the first order and the resultant respiratory quotient approaches unity. Time relations are now such that the conversion of fat to carbohydrate of a velocity, say of the second order, has no effect upon the respiratory quotient. The quotient will remain high until the glycogen is considerably lowered. The depletion of readily available glycogen results in speeding up the reaction fat to glycogen, or whatever other processes lead to the utilization of other substances, and the quotient reflects this reaction. The authors stated that the strongest argument in favor of the view that glycogen is the invariable source of muscular energy seemed to them to depend upon the consideration that the intrinsic physico-chemical processes involved in muscular contraction are always the same. Hence, it seems probable that whatever different sources may involve the process of muscular contraction, the chemical process through which all substances pass must be in part common to all of them. They believe that glycogen or some substance

very closely related to it in normal metabolism is the immediate source of the energy of muscular contraction.

The most extensive series of experiments on humans in the study of the respiratory quotient of excess metabolism of exercise was made by Best, Furusawa, and Ridout (1929) over a period of two years. One group of experiments was made in Toronto, another in Ithaca, and the third in London. The authors prescribed the following precautions for obtaining reliable and significant results. The period of rest should not be less than thirty minutes during which the mouthpiece or similar apparatus should be worn, *i.e.*, breathed through. They advise collecting the whole of the expired air in one bag or suitable container, or if collection is made in several portions, preventing escape of any of the expired air. The period necessary for recovery cannot be guessed but must be determined by experiment. Unless recovery is known to be complete, the respiratory quotients have no meaning except in so far as they show the production and removal of acid in the body. A practiced and suitable subject should be used. The authors state that to employ a subject showing irregular results is not to prove that any foodstuffs may be used, but rather a sign of insufficient experimental precautions.

The first group of 23 experiments was made at Ithaca, New York, on sprint running at absolutely top speed. The excess respiratory quotients varied from 1.18 to 1.68 and in the majority, the respiratory quotient of the total metabolism of activity and recovery was nearly unity. They consider two possibilities for the causes of the results, first, that excess carbon dioxide was blown off with consequently too short a recovery process, second, that the resting metabolism had been changed as the result of violent exercise, and that more carbohydrate was used during the basal oxidations proceeding at the same time with recovery process than during the same oxidation before exercise. If the first suggestion were correct, they would expect a retention of carbon dioxide in a later period of recovery which should lead to a lower respiratory quotient in the second resting determination. There is some evidence of a lower final quotient in 11 experiments, but the quantity of carbon dioxide still being retained was far too small to compensate materially for the large quantity which had been expired in excess. Moreover, in the other experiments the respiratory quotient in the second rest period was equal to or higher than that of the first. They were dealing with such large excesses of carbon dioxide that the small fall of 6 cc. per minute would require several hours for the retention of the extra carbon dioxide which had to be accounted for. In order to test the question

as to whether it was a washing out of carbon dioxide, they had several sprint runs with recovery periods in between each pair of runs. The results were the same as when only one run was made. Excess carbon dioxide was found after the second and after the third run, and it seemed therefore inconceivable that the extra carbon dioxide was due to excessive respiration and acid formation. The total extra carbon dioxide to be accounted for would make an excess of about 10 liters, the equivalent as an acid of 40 grams of lactic acid. This amount of lactic acid over and above that found normally at rest would cause a rise in the blood and soft tissues to more than 110 milligrams per cent, a value which is about the maximum found after exercise.

Another group was obtained by Furusawa in London with standing running at an extreme speed of 260 steps per minute. Forty to 60 minutes were allowed for the collection of expired air after exercise. The respiratory quotient of the total metabolism was about unity and the excess metabolism had a respiratory quotient of 1.19 to 1.68. Further experiments at Toronto by Best and Ridout confirmed these results. The time allowed for recovery was 50 minutes.

In order to determine whether there was a washing out of carbon dioxide at any stage with a subsequent retention, the recovery period was divided into several short periods. There was, however, little evidence of a retention of carbon dioxide or fall of respiratory quotient. In the blood and tissues lactic acid may displace an equivalent amount of carbon dioxide. The calculated quantities of extra carbon dioxide were from 1.8 to 2.5 liters with a corresponding expected presence of 7.3 to 10.6 grams of lactic acid supposedly at the end of an hour's recovery from exercise lasting only half a minute. These are impossible amounts. In addition, the same process could be repeated again and again. A direct determination of the lactic acid content after exercise and recovery proved that there was no important increase in the lactic acid content of the blood.

A group of 7 experiments with more moderate activity was conducted at Ithaca, and showed that the respiratory quotient of the excess was very close to unity. Similar observations made in London confirmed this series and a group at Toronto on standing running at moderate speed, and with riding a bicycle ergometer at slow speed, showed essentially the same results.

A group of 29 experiments with standing running at low speed stepping low in Toronto showed consistently that the excess respiratory quotient

was lower than unity. In fact, some of the values were nearly equal to the basal respiratory quotient.

The authors stated that it might be assumed that the excess metabolism is only such a small fraction of the total metabolism in the case of mild exercise that small errors in the estimation of the quantities involved might lead to erroneous results. The large number of observations, however, and their consistency seemed to weaken this objection. There was a difference between the two subjects as to the level of exercise at which the respiratory quotient rose to unity. In the case of C. H. B., an oxygen requirement of 1.0 to 1.9 liters per minute showed the lower level of the excess respiratory quotient, whereas, E. Mc H. exhibited a value nearly unity at an oxygen requirement of 0.7 liter per minute.

A group of experiments was conducted by Furusawa in London with moderate exercise with values varying from 0.74 to 1.04 liters of oxygen for the excess metabolism. According to the authors, where the oxygen requirement of exercise was less than 0.5 liter per minute, a small error in the resting metabolism of 2 or 3 cc. per minute could have made a large error in the respiratory quotient of the excess metabolism. Experiments were performed with longer resting collection of from 40 to 60 minutes, but this made no difference in the results.

The authors conclude that the high excess respiratory quotient was really caused by some metabolic change induced by very violent exercise. The results showed clearly that in the case of mild or very mild exercise, carbohydrate is not the sole foodstuff involved in muscular exercise. The respiratory quotient of the excess metabolism accompanying exercise did not invariably attain the value of unity. It is a function of the oxygen requirement rising gradually from the level of the basal metabolism to a very high value. It seems impossible to explain this gradually changing nature of the excess metabolism, especially the high values observed, by any such simple assumption as the combustion of carbohydrate and the washing out of carbon dioxide, or by the hypothesis that the body can utilize any foodstuffs available in order to provide energy for muscular contraction. They then discuss the possibility of an excess mobilization of sugar with a subsequent conversion to fat. However, they did not obtain any evidence of a liberation of sugar in their experiments and they did not, therefore, feel justified in this explanation.

They state that the small increase observed in the total respiratory quotient in very moderate exercise indicated that the body was able to speed up the preparation of carbohydrate from other materials to meet the in-

creased demand. In this case, the new formation could keep pace with the rate at which the muscles consume carbohydrate and the effect on the respiratory quotient is that any foodstuffs could be used directly to provide energy for muscular contraction. With increased severity of exercise the demand for carbohydrate increased and the process of new formation was no longer adequate to meet the demand. The increased combustion of carbohydrate may then overshadow the effect due to the contemporary manufacturing process. Consequently the stage at which the respiratory quotient of the excess metabolism reached unity was arrived at, and finally extreme violent exercise induced a totally new phenomenon for which no satisfactory explanation exists.

Their investigation is the most complete series of measurements on the metabolism of exercise and recovery thus far available. It has the great advantage that the several series were conducted in three different places by different observers and therefore there is no question involved of the use of one instrument throughout or only one observer. The group of experiments in which the respiratory quotient of the excess metabolism was over unity form the marked exception to all other investigations. It would seem to be most important for this type of study to be repeated by other observers. At present there seems to be no satisfactory explanation for this phenomenon.

Carpenter and Fox (1929) determined the respiratory exchange of a subject who rode on a Krogh ergometer for one hour at 275 kg-m. per minute and for one-half hour at 555 kg-m. per minute. The work period was preceded by a base line measurement of four 15-minute periods in succession and the periods of work were followed by seven 15-minute periods of recovery after the one hour's work and nine 15-minute periods of recovery after the one-half hour's work. The measurement of the expenditure due to work included the act of rising from a chair, mounting the ergometer, and the recovery periods included dismounting the ergometer and returning to the chair. The measurement of respiratory exchange during the work and recovery periods was continuous and was made with an open circuit apparatus with which a mouthpiece and valves were used. The respiratory quotients of the base line for the two groups varied from 0.78 to 0.87. The oxygen absorption during the periods was approximately 1000 cc. per minute in the one hour's work and in the one-half hour's work was approximately 1500 cc. In the one hour's work, the average respiratory quotient of the excess metabolism was 0.89 with a variation from 0.93 to 0.86 and for the one-half hour's work, 0.95. Thus in both groups the metabolism of

excess was higher than the base line and in the case of the more intense work of one-half hour was not far from unity. Two groups of experiments with the same amount of work and conditions of measurement were made in which 50 grams of glucose were given just before beginning the work. The respiratory quotients in the base line periods averaged 0.83 for the one hour's work and 0.82 for the one-half hour's work. The respiratory quotients for the excess metabolism averaged 0.96 and 0.99. When the excess gaseous metabolism was corrected for the increases in carbon dioxide and oxygen absorption due to the ingestion of 50 grams of glucose at rest, the respiratory quotient of the corrected excess metabolism was 0.94 and 0.98, thus higher than the excess quotients obtained when no sugars were given under the same conditions of work and recovery. Therefore, when glucose was given simultaneous with work, at least part of the ingested glucose was utilized in the performance of work.

These experiments differed from most work of similar nature by others in the long basal period. Care was also taken that the subject would be in a position comfortable enough so that he could relax completely after the work was done.

The results obtained by various investigators vary considerably. Some of these variations must be ascribed to the conditions under which the experiments were performed. One of the most important measurements is that of the basal metabolism or base-line both from the standpoint of total oxygen absorption and from the standpoint of the respiratory quotient. The conditions of the subject with respect to posture, bodily comfort, and possibility of complete relaxation under which he is measured should be the same as those that he is likely to reach after the work and recovery periods are finished. It must be obvious that a measurement of the R.Q. made after a shift in position without a sufficient length of time for adjustment cannot be considered reliable. The practice of having the subject breathe through the breathing appliance during at least one quarter hour and preferably the whole of the rest period before a measurement is made is a good one. This gives opportunity for adjustment of the organism to the new conditions of respiration and the subject is more likely to give a true respiratory quotient as well as normal oxygen absorption after a breathing period than after insertion of the mouthpiece within two or three minutes of taking a position. It would be advisable to have more than one period, if possible, in succession without an interval, because then there is no opportunity for compensation. There does not seem to be any reason for having the exact amount of work performed as one can obtain an estimate upon

this from the oxygen requirement. The condition to which the subject will return after the work is finished should be one in which it is possible to measure the respiratory exchange indefinitely or at least for two or three hours. The measurement of a recovery period while the subject is sitting upon a bicycle ergometer does not seem to be a practical one so far as complete relaxation is concerned, and it would seem as though it was necessary to continue the recovery period until the oxygen absorption had returned to the pre-work value or to a base line which is constant for several periods in succession. If the work has been so intense and stimulating as to cause a rise in metabolism which persists for some time, it is obvious that one cannot expect a return to the pre-work level in the oxygen absorption. Some investigators have laid down the condition that the recovery period should end when the pre-work respiratory quotient has been attained. This, however, must of necessity come within a very short period after the work finishes as the fall to a low quotient is rapid in the recovery period after work is finished. Subsequently there may be a rise which probably more truly represents the actual respiratory quotient after recovery is complete than the point at which the respiratory quotient reaches the pre-work level for the first time. If the work has been continued long enough and is exhausting enough, it is obvious that the respiratory quotient may not return to the pre-work level again until food is furnished, and therefore there cannot be a return of the carbon dioxide as well as oxygen to the pre-work level.

The technique of the determination and the physiology of the respiratory quotient has recently been treated very thoroughly by Richardson (1929).

The best apparatus for measuring the respiratory exchange and determination of the respiratory quotient is one of an open circuit type in which the outgoing air is analyzed by some form of Haldane apparatus, if the breathing applicance is a mouthpiece, and by the gas analysis apparatus of Carpenter (1931), if it is a helmet device such as recently described by Benedict (1930). The helmet undoubtedly furnishes the optimal conditions for normal breathing and it is possible to continue measurements of the respiratory exchange over long periods of time with little or no discomfort to the subject. The closed circuit principle for such types of work does not seem the most suitable, either with a chamber or with a breathing appliance. The difficulties of the determination of the respiratory quotient by the chamber method were pointed out by Carpenter (1915) who also came to the conclusion that the most logical method for the determination of the respiratory quotient with a breathing appliance was by the analysis

of expired air. Krogh and Lindhard (1920) made an analysis of the results obtained in the above publication (Carpenter, 1915) and chose the open circuit chamber apparatus for their extensive study of the respiratory exchange of muscular work. Benedict (1925) has recently stated that the open circuit arrangement with a chamber is the ideal method for the determination of the respiratory quotient.

The method of determining the total gaseous exchange during muscular activity and recovery and then calculating the respiratory quotient of the excess above the resting level has furnished a new point of attack on the problem of the source of energy for muscular work, but one must never lose sight of the fact that the excess respiratory quotient is a result of calculation and not the result of direct determination. It is not improbable that one may attach too much significance to this calculated ratio. All sorts of absurd ratios of excess gaseous exchange may be obtained if the suitable conditions are provided. An increase in carbon dioxide at rest with no increase in oxygen absorption will result in a calculated ratio of excess of infinity. So that one must be on guard against ascribing too much significance to ratios of excess gaseous exchange. A more correct perspective would be obtained from a study of the changes in total metabolism and total proportion of nutrients than from the metabolism and nutrients of the excess gaseous exchange due to muscular activity.

Various interpretations have been given to the results obtained by the different authors. It is evident that the proof is not adequate that carbohydrate is used exclusively in muscular activity of man. On the other hand, it does not seem evident that the nutrients used during muscular activity are used in the same proportion as they are during rest. Some of the experiments point in this direction but a general assessment of all of the results indicates that under certain conditions the proportion of carbohydrate used is greater during muscular activity than during rest. With very light moderate work of short duration there seems to be but little difference between the mixture metabolized during rest and that burned during work. On the contrary, when the work is increased, particularly in intensity, there is a general tendency toward an increase in the proportion of carbohydrate catabolized. When the work is continued and is more intense, the available carbohydrate seems to become diminished and then there is a call which results in a catabolism of fat with a lowering of the respiratory quotient. This catabolism is not necessarily a transformation of fat to carbohydrate with a subsequent catabolism of the latter.

The reviewer's hypothesis with regard to the fuel of muscular activity

is as follows: It would seem as though with extremely light work the available metabolites which result from the mixture of nutrients at rest were first used. These may be conceived of as small molecules from carbohydrate, protein, and fat. When the metabolites from fat which are easily oxidized are disposed of, the carbohydrate would be drawn upon, because this may be the more easily metabolized. Subsequently the available type of carbohydrate becomes diminished and then there is a diminishing amount of carbohydrate metabolized with an increasing amount of fat. It seems to the reviewer that some sort of combination, such as the above, may explain the lack of change in the respiratory quotient when the work is very moderate, the increasing carbohydrate utilized when the work is increased, and the drawing upon fat when the work is continued. This conception is somewhat like that of Bock and associates (1928) except that there is no implication of conversion of fat to carbohydrate. Such an hypothesis does not, however, explain the extraordinarily high quotients found with very intense work by Best, Furusawa, and Ridout. In fact, they are unexplainable at the present time.

In connection with recent work upon the source of energy for muscular activity very little or no consideration has been given to the rôle of ethyl alcohol in muscular activity. The literature on this subject is very conflicting, so that the question as to whether the energy of alcohol is convertible in muscular activity in the animal organism is by no means settled. It would seem that it would be worth while to reinvestigate the subject with present methods of study and points of view (excess respiratory quotient, formation and disappearance of lactic acid). It is evident that, if the availability of the energy of alcohol for the performance of muscular work is demonstrated, recent theories and conceptions of the chemistry of the metabolism of muscular activity would have to be revised. The use of ethyl alcohol as compared with simple sugars would also lead to evidence as to whether the size and availability of the molecules of nutrients or metabolites were correlated with their availability as sources of energy for muscular work.

It does not seem as though lactic acid were an essential member in the chain, although there is not the slightest doubt that lactic acid is formed in large amounts in muscular work, but it has been shown by several workers (Martin, Field, and Hall, 1929; Gollwitzer-Meier and Simonson, 1929) that the oxygen recovery curve and lactic acid disappearances are not parallel. The lactic acid remains a long time after the oxygen absorption has reached the pre-work level. Also, attention must be called to the fact

that the lactic acid-glycogen cycle exists during work as well as during recovery and that the liver is removing lactic acid from the blood even during muscular work (Himwich, Koskoff, and Nahum, 1930).

The studies of Eggleton and Eggleton (1927a, 1927b, 1928) on "phosphagen," the separation and identification of phosphocreatine by Fiske and Subbarrow (1927, 1929), and the investigations of Lundsgaard (1930a, 1930b) on muscular contraction without lactic acid formation have shifted the interest in the chemistry of muscular activity for the time being to another point of attack, and have led to questioning of the rôle of lactic acid in the chemical and energy transformations in muscles. However, not all of the adherents to the lactic acid theory have lost their faith, as Hill (1931) has recently expressed the opinion that lactic acid will "come back" if for no other reason than because of the far greater energy at its disposal than that of phosphagen. As stated at the beginning of this review, from time to time new investigations shift our interest. Will they remove completely the older conceptions and the older methods of attack or will they supplement, amplify, and explain results obtained?

T. M. C.

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BASAL METABOLISM STANDARDS

A STATISTICAL COMPARISON OF THEIR PREDICTION VALUES

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THE present study was undertaken in an effort to improve the prediction value of basal metabolism standards. This effort was unsuccessful, in that, as will be presently shown, no significant improvement was realized; but in the process a statistical comparison of the prediction value of various standards now in use was made. The purpose of this article is to present the results of this comparison, and to stress certain principles in the comparison of standards overlooked by several previous writers.

Comparisons of predicted basal metabolism with that obtained by experimental measurement have commonly been made by calculating the average deviation of the observed metabolism from the predicted metabolism. This practice has been a most unfortunate one and has contributed confusion rather than clarity to the problem. It violates what appears to the writer as a fundamental principle of basal metabolism standards—that these standards are to be regarded as relative and not absolute. Apparently climate and race influence the basal metabolism. Be the cause what it may, the determination of basal metabolism in various laboratories gives results which frequently parallel each other at different levels. The calculation of *average deviation* does not in any way distinguish between prediction errors which are a result of the failure of the predicted values to *parallel* the observed values, and those which are the result of a difference in the *level* regarded as normal. To take a hypothetical, extreme example, let us assume that we have 100 normal men whose metabolism has been determined and expressed in terms of standards A and B. According to standard A, which was worked out in the laboratory in which these men have been studied, the basal metabolism of these men ranges from minus 12 per cent to plus 12 per cent with an average deviation of let us say 6 per cent. According to

standard B which was perhaps worked out in a somewhat colder climate, the basal metabolism ranges from minus 11 per cent to minus 9 per cent with an average deviation of 10 per cent. By the comparison of average deviations, the former standard would be the better. Actually the latter

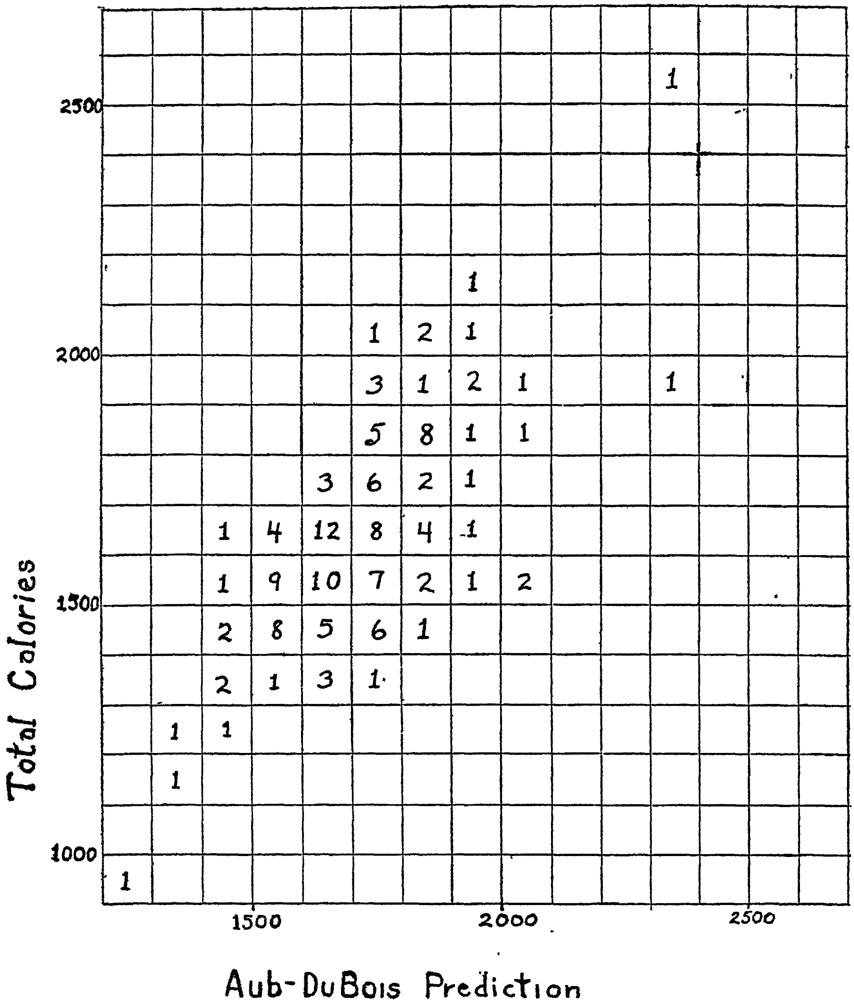

FIG. 1

gives a much more accurate relative prediction, and if 10 per cent were added to each determination, it would give a far more accurate absolute prediction. It is not necessary in clinical use to add the 10 per cent, but simply to revise one's view of what is normal metabolism in this locality as determined in this laboratory according to standard B.

This principle may be stated as follows: *the empirical test of a basal metabolism standard is its accuracy in the relative prediction of the basal metabolism of normal human beings.* Its corollary is; *for the clinical applica-*

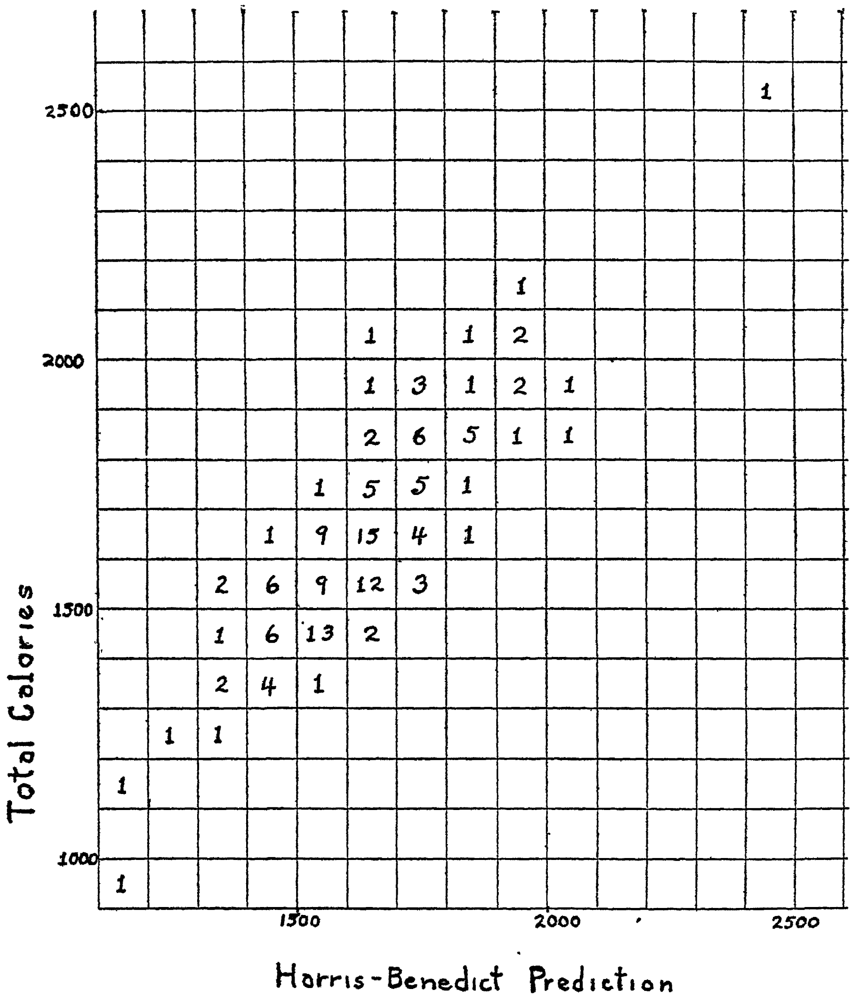

FIG. 2

tion of any basal metabolism standard there should be available the mean basal metabolism of normal individuals of the region, determined under the conditions used for clinical determinations. The above principle was recognized by Stoner (1) in his criticism of Boothby and Sandiford (2). The latter writers compared the metabolism of 127 normal people as determined by the Harris-Benedict and Aub-DuBois standards. They found 92 per cent

of the determinations within ±10 per cent by the Aub-DuBois standard, and only 82 per cent by the Harris-Benedict. As Stoner points out, this is because the *mean* of the Aub-DuBois is more nearly coincident with the *mean* of the normal as determined at the Mayo clinic, than is that of the Harris-Benedict standards. Eighty-nine per cent of the patients fall within plus 14 per cent and minus 6 per cent by the Harris-Benedict standards.

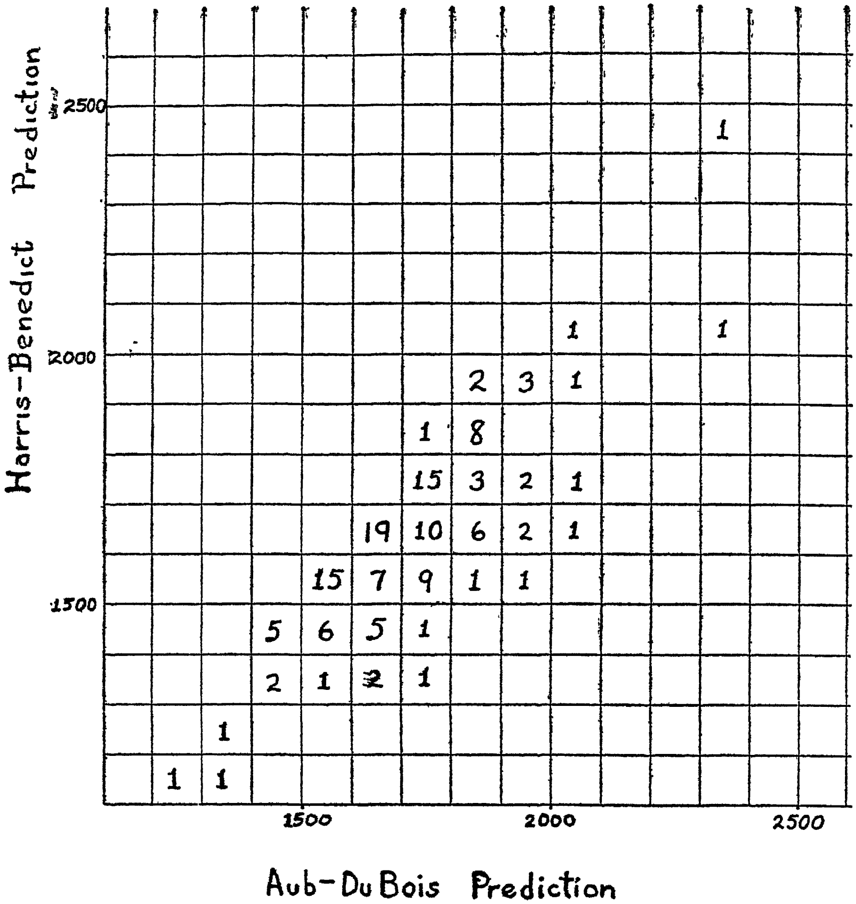

FIG. 3

Two methods are available for the comparison of relative prediction values with facility. One is the comparison of scatter diagrams of predicted caloric production with those obtained by calorimetry, direct or indirect, and the calculation of correlation coefficients. The other is the calculation of the standard deviation of errors of prediction.

Standards for Adult Males

Figures 1, 2 and 3 are scatter diagrams of observed and predicted caloric consumption based upon the data on males published by Harris and Benedict (3) in their monograph. Table I lists the Pearson product-moment coefficients of correlation. It will be noted that the Harris-Benedict prediction formula is not only superior to the Aub-DuBois on these data, but that the correlation between it and the determined metabolism is greater than that between the two standards.

It will be noted that as might be expected on these data the mean predicted value by the Harris-Benedict standard is much closer to the mean of the observed determinations than is that of the Aub-DuBois standard. The standard deviation of the actual caloric consumption is also, as might be expected, distinctly greater than that of either standard.

Table I

Pearson product-moment correlation coefficients	
Harris-Benedict standard and observed metabolism	$r=.84\pm.031$
Aub-DuBois standard and observed metabolism	$r=.69\pm.042$
Harris-Benedict standard and Aub-DuBois standard	$r=.80\pm.035$
Mean value, calories	
Observed	$M=1635$
Predicted, Harris-Benedict	$M=1631$
Predicted, Aub-DuBois	$M=1712$
Standard deviation, calories	
Observed	$\sigma=210$
Predicted, Harris-Benedict	$\sigma=176$
Predicted, Aub-Dubois	$\sigma=168$

It must be borne in mind that these comparisons are being made on the data from which the Harris-Benedict standards were developed, and that therefore the Harris-Benedict standards are at an advantage compared with the Aub-DuBois standards in that they may be expected to fit this particular body of data exceptionally well. This is also true of the Dreyer standards, and to a lesser extent of the two new formulae presented here, in that these were based upon a portion of the cases presented by Harris and Benedict, or in part based upon them.

In order to make a more extensive comparison of standards, the predicted caloric consumption in 24 hours by each of a series of standards was expressed in terms of the observed consumption. This is the reverse of the usual procedure. Inasmuch as this investigation is being made of the *standards* instead of the metabolic rate of particular cases, it is the more logical method here and was also the simpler procedure in mass calculations. The mean and the standard deviation of each series of percentages

were then calculated. Inasmuch as a standard deviation of 5 per cent does not have the same quantitative significance with a mean of 110 per cent as it has with a mean of 95 per cent, it was decided to correct all standard deviations to a mean of 100 per cent by dividing each by the corresponding mean. This gives a figure which, if one drops the per cent sign, is numerically equal to the coefficient of variation.[1] The results are tabulated in Table II.

TABLE II

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF HARRIS AND BENEDICT
(Men only, 134 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	6.10 ± .250	±4.11	100.4 ± .36	−5.6
Power formula for adults	6.15 ± .252	±4.14	99.5 ± .36	−6.5
General power formula	6.46 ± .266	±4.36	100.5 ± .37	−5.4
Dreyer (observed weight)	6.58 ± .270	±4.44	99.4 ± .38	−6.5
Mayo	8.28 ± .341	±5.58	108.2 ± .52	+2.3
Bailey	8.55 ± .352	±5.77	106.6 ± .53	+0.7
Aub-DuBois	8.59 ± .353	±5.79	105.9 ± .53	+0.0
Krogh	8.64 ± .354	±5.83	97.7 ± .50	−8.3

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

From the corrected standard deviation, the corrected probable error was obtained by multiplying by .6745. The formulae and standards are arranged in order of their relative prediction accuracy—inversely as the magnitude of their standard deviations. The Harris-Benedict standard (based upon the Harris-Benedict tables) gives slightly the best relative prediction. The author's power formula for adults (78.36 Age $^{-.142}$ Ht. $^{.214}$ Wt. $^{.575}$) runs an insignificant shade behind. The writer's general power formula, for both adults and children (6.77 Age $^{-.122}$ Ht. $^{.331}$ Wt. $^{.345}$) comes third, followed by the Dreyer standard based on observed weight (4) (calculations based upon the tables published by Stoner) (5, 6). There is then a significant

[1] Whereas the coefficient of variation has a probable error very complex to calculate, (because account is taken of the error of the mean as well as the error of the standard deviation) the probable error of the standard deviation is very simple to calculate, being .6745 $\sigma/2N$ and can be corrected by dividing by the mean in the same fashion as the standard deviation itself. The result is a figure spuriously but negligibly lower than the probable error of the coefficient of variation. The corrected standard deviation was used in place of the coefficient of variation because of the simplicity of the calculation of its probable error.

drop in prediction value to the standards based upon the DuBois height-weight formula (7) for surface area. (The surface area was taken from the DuBois table.) Of these, the standard (8) published by Boothby and Sandiford, called here the Mayo standard for convenience, gives slightly the best result, followed by the Bailey standard (9), the original Aub-DuBois (10) and The Krogh standards (11) in order.

A considerable variation is to be noted in the mean of the various standards. The lowest standard (which, of course, would tend to give the highest rates) is that of Krogh. Less than 2 per cent higher is the Dreyer standard, and the Harris-Benedict is about 1 per cent higher still. There is then a distinct jump to the Aub-DuBois standard which is 8 per cent above the Krogh and 5.5 per cent above the Harris-Benedict. Next comes Bailey's standard and highest of all is the Mayo standard, over 2 per cent above the Aub-DuBois. There is thus a 10.5 per cent difference between the Krogh standard and the Mayo standard. This type of difference must not be lost sight of, as it is half the width of the usually accepted "normal" range.

TABLE III

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF HOBSON
(Males, 46 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Bailey
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	6.10±.429	±4.11	94.9±.58	−5.2
Mayo	6.26±.440	±4.22	101.4±.63	+0.3
Dreyer (calculated weight)	6.50±.458	±4.39	97.5±.63	−2.6
Hobson's modification of Dreyer's formula	6.55±.461	±4.42	100.6±.66	+0.5
Bailey	6.62±.465	±4.47	100.1±.66	0.0
General power formula	6.79±.478	±4.58	93.5±.63	−6.6
Power formula for adults	6.81±.478	±4.59	93.5±.63	−6.6
Harris-Benedict	7.19±.506	±4.85	94.0±.67	−6.1

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

If we turn now to other data for a comparison of standards, we find no comparable body of published cases. Hobson (12) published the results of the examination of 46 males, ranging in age from 9 years to 40. The results of study of this group are summarized in Table III, the Krogh and the DuBois standard being omitted because of the age range. None of the differences of standard deviation is significant, but Dreyer's standard based

on observed weight gives the best agreement. The relatively poor agreement given by the standards based upon the body surface law in the data of Harris and Benedict is not apparent here. The mean values are not

TABLE IV

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BOOTHBY AND SANDIFORD
(Men only, 41 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	5.95±.443	±4.00	94.8±.59	−4.4
Dreyer (observed weight)	5.96±.444	±4.02	94.3±.59	−4.9
Mayo	5.86±.436	±3.95	99.7±.62	+0.4
Krogh	6.14±.457	±4.15	93.1±.60	−6.0
Aub-DuBois	6.19±.462	±4.17	99.2±.65	0.0
Bailey	6.19±.462	±4.17	100.2±.65	+1.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE V

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF EARLE
(Chinese men, 87 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	6.59±.337	±4.44	107.6±.51	−2.5
Dreyer (observed weight)	6.85±.351	±4.62	109.8±.54	−0.3
Krogh	7.10±.363	±4.79	105.1±.54	−5.0
Aub-DuBois	7.13±.364	±4.81	110.1±.57	0.0
Bailey	7.20±.368	±4.86	110.9±.58	+0.8
Mayo	7.26±.371	±4.90	113.4±.60	+3.3
Dreyer (calculated weight)	7.70±.394	±5.20	110.0±.61	−0.1

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

widely changed with relation to each other, but these cases consumed relatively more oxygen as a group, than did the previous ones. It should be noted that the substitution of calculated weight for observed weight in Dreyer's formula and the modification of the formula made by Hobson to fit these particular cases both actually diminish the relative prediction

value of the formula. No evidence is forthcoming that there is any advantage in either of these modifications, over Dreyer's original formula. Inasmuch as the Harris-Benedict published tables do not cover the age range in this table (although the writers say the formula is applicable) it was necessary here and occasionally later to make calculations directly from the formula.

In Table IV are entered the data obtained by a comparison of the forty-one normal males published by Boothby and Sandiford (13). The general level of calorie consumption is greater in this body of data than in either of the previous ones. This is reflected in the high level of the Mayo standards published by Boothby and Sandiford.

TABLE VI

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF AUTHOR
(College freshmen, men only, 34 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	7.04±.576	±4.75	102.4± .83	−5.6
Dreyer (observed weight)	7.11±.582	±4.79	101.5± .84	−6.5
Krogh	7.84±.643	±5.29	100.9± .92	−7.1
Mayo	7.90±.648	±5.33	109.5±1.00	−1.5
Bailey	8.08±.662	±5.45	107.4±1.01	−0.6
Aub-DuBois	8.27±.677	±5.58	108.0±1.03	0.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

Further data are treated in Tables V and VI. Table V combines series A, B, and C published by Earle (14). These are all determinations upon Chinese men in Hongkong and seem comparable. It will be noted that again the Dreyer standard based upon calculated weight is inferior to the Dreyer standard based on observed weight. As Boyd (15) has pointed out, calculating body weight from sitting height (and one might certainly add, from chest circumference) means the substitution of a less accurate determination (sitting height or chest circumference) for a more accurate one. Table VI contains data on 34 male university freshmen from determinations by the author.

The Harris-Benedict formula gives on the whole the best relative prediction on the data available on men, with the Dreyer standard (observed weight) and the Mayo standard following.

Standards for Women

The tabulation of data from the material of Harris and Benedict (3) on women is contained in Table VII. Here there are no significant differences in relative prediction accuracy, although the Dreyer (observed weight)

Table VII

Prediction Value of Various Standards on Data of Harris and Benedict
(Women only, 103 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	7.68±.361	±5.17	99.8±.51	−4.8
Krogh	7.72±.363	±5.20	98.6±.51	−6.1
Mayo	7.78±.366	±5.24	103.3±.53	−1.3
Aub-DuBois	7.82±.367	±5.27	104.7±.54	0.0
Harris-Benedict	7.85±.369	±5.29	100.8±.33	−3.8
Bailey	8.21±.386	±5.54	105.0±.57	+0.4

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

Table VIII

Prediction Value of Various Standards on Data Of Boothby and Sandiford
(Women only, 61 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Bailey	5.02±.306	±3.39	100.8±.44	+0.5
Krogh	5.46±.333	±3.68	94.2±.44	−6.1
Mayo	5.55±.339	±3.74	99.1±.48	−1.2
Aub-DuBois	5.59±.341	±3.77	100.3±.48	0.0
Harris-Benedict	5.85±.357	±3.95	96.3±.49	−4.0
Dreyer (observed weight)	6.17±.377	±4.16	95.6±.51	−4.7

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation. ($100\sigma/M$) divided by 100. It has a different probable error.

prediction is slightly the best on this particular body of data. The differences in mean level roughly parallel those found in the data of these authors on men.

In Table VIII is the material on normal women (61 cases) published by Boothby and Sandiford. Here the Bailey prediction is the best, but none of

the differences is by itself significant. The comparison on the relative levels of the standards agrees accurately with that on the previous body of data.

The standards built on the DuBois height-weight surface area formula give the best agreement with the data for women, the Krogh standard coming second, and the Mayo third.

Standards for Children

Standards for children are in an even more confused and divergent condition than standards for adults. In Table IX is listed the comparison of

TABLE IX

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BENEDICT AND TALBOT COMMON TO ALL (Boys, 27 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Benedict-Talbot weight (interpolated)	6.01±.551	±4.05	100.7±.78	0.0
Dreyer (observed weight)	6.15±.564	±4.15	113.0±.90	+12.3
Benedict-Talbot weight (as published)	6.19±.568	±4.18	100.3±.81	− 0.4
Power formula for children	6.24±.572	±4.21	97.2±.79	− 3.5
Harris-Benedict	6.50±.596	±4.38	97.7±.82	− 3.0
Mayo	6.75±.619	±4.55	112.6±.99	+11.9
General power formula	6.78±.622	±4.57	99.6±.88	− 1.1
Bailey	7.39±.678	±4.98	118.8±1.14	+18.1
Kestner-Knipping	7.80±.716	±5.26	110.9±1.13	+10.2

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

the standards on all boys over 5 years of age and over 25 kilograms weight, in the data of Benedict and Talbot (16). The age limit was required by the Mayo, Bailey and Dreyer standards, and the weight limit by Stoner's table of the Dreyer standard (6), which does not extend below 25 kilograms weight. When the Benedict-Talbot weight standard (16) is interpolated by tenth of kilogram steps, its accuracy is slightly increased. The author's power formula for children (2311 Age $^{.304}$ Ht. $^{-.638}$ Wt. $^{.484}$) gives a slightly less accurate prediction than the Benedict-Talbot weight standard.

Table X contains the comparisons on the data of Rosenblüth (17). In all of the tables here published on children, successive determinations on

the same child at intervals of more than a year are treated as separate cases. The standard of Dreyer is here the best, and the Nobel-Rosenblüth (17) is included merely to show how bad it is. The Kestner-Knipping (18)

TABLE X

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF ROSENBLÜTH
(Boys, 11 years to 19 years, 84 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	6.17±.321	±4.16	89.8±.45	+4.5
Mayo	6.68±.347	±4.50	95.2±.49	+9.9
Bailey	6.87±.358	±4.64	94.5±.51	+9.2
Benedict-Talbot weight	7.34±.381	±4.95	85.3±.54	0.0
Kestner-Knipping	7.54±.393	±5.09	91.4±.56	+7.8
Nobel-Rosenblüth	8.96±.466	±6.04	83.6±.66	−1.7

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE XI

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BENEDICT AND TALBOT COMMON TO ALL EXCEPT DREYER (Girls, 25 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Benedict-Talbot weight (interpolated)	5.30±.505	±3.57	102.0±.73	0.0
Benedict-Talbot weight (as published)	6.04±.576	±4.07	101.4±.83	− .6
Benedict height	6.06±.578	±4.08	99.9±.82	−1.6
Kestner-Knipping	6.38±.608	±4.30	117.1±1.01	+15.6
Mayo	6.48±.618	±4.37	119.9±1.05	+17.9
Bailey	6.60±.629	±4.45	116.6±1.04	+14.6

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

standard shows up poorly on both tables. We must regard the Dreyer as the best standard for boys, with the Mayo standing second, if we accept this evidence.

Tables XI, XII (19) and XIII (20) contain comparisons of the available

data on girls. The Mayo, Bailey and Dreyer standards stand out as the best, in this material. The wide differences of mean level (about 20 per cent on each table) should be noted.

TABLE XII

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BLUNT, TILT, McLAUGHLIN AND GUNN
(Girls, 7 to 18 years, 54 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Mayo	7.43±.484	±5.01	104.0±.68	+ 9.6
Bailey	7.45±.485	±5.02	108.3±.69	+13.9
Kestner-Knipping	8.42±.548	±5.68	101.8±.77	+ 7.4
Dreyer (observed weight)	8.60±.580	±5.80	99.9±.82	+ 5.5
Benedict-Talbot weight	9.82±.640	±6.62	94.4±.90	0.0
Benedict height and Benedict age and weight	10.46±.681	±7.01	91.0±.96	−3.4

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE XIII

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF MACLEOD
(Girls 10 to 14, 63 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot
	Per cent	Per cent	Per cent	
Mayo	10.92±.657	±7.37	111.3±1.03	+15.7
Dreyer (observed weight)	10.98±.661	±7.41	106.6±1.00	+11.0
Bailey	11.21±.674	±7.56	115.9±1.10	+20.3
Kestner-Knipping	11.49±.691	±7.75	105.3±1.03	+ 9.7
Benedict-Talbot	12.61±.758	±8.50	95.6±1.03	0.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different porbable error.

THE SELECTION OF NORMAL STANDARDS

There are here presented three power formulae for the prediction of normal basal metabolism, one for use on adult males, one on male children of two years and over, and a third which endeavors to predict the basal metab-

olism of both adult males and male children. Inasmuch as he has been able to demonstrate no definite advantage of these formulae over those in use, the writer regretfully advises no one to adopt these formulae as standards. Since these formulae are but typical of an almost unlimited number of possible other formulae of about the same prediction value he would respectfully suggest to other similarily ambitious individuals that they also restrain themselves until such time as they are able to present statistically significant data to the effect that the new formula marks a real advance in prediction accuracy.

There are several criteria for the selection of a normal standard. The first obviously is prediction accuracy, which will be *relative* prediction accuracy. A second is range of applicability, in that one standard is preferable to several. A third criterion is simplicity of application. Then also, since uniformity is desirable, there is a certain seniority of accepted and established standards to be considered. Still another desirable feature for a basal metabolism standard is approximate agreement in regard to the mean metabolic rate for comparable groups of normal men and women.

In the opinion of the writer, the Dreyer standards based upon observed weight meet the criteria outlined, on the whole, the best. They are the simplest of application in that a single table (5, 6) can be used and the measurement of height is rendered unnecessary. They have a wide range of applicability (from 5 years of age on). They have, on the whole, the best relative prediction accuracy of any standard on the material presented. They have a degree of established usage, particularly in England. The substitution of calculated weight for actual weight in the Dreyer standard is to be condemned. This practice has no observable advantage. According to the available evidence it noticeably diminishes the relative prediction accuracy of the Dreyer standard, as might be expected from the fact that it substitutes inaccurate measurements for accurate ones. It sacrifices the simplicity of the Dreyer standard, and makes it the most complicated of all, and by this token increases the likelihood of errors of calculation.

The Mayo standards are the nearest competitors to the Dreyer standards, but are more complex. They have equal range of applicability, and while not widely used at present, are built upon the DuBois height-weight formula for body surface, which has a wide usage.

The Harris-Benedict standards give excellent agreement results for adults, but necessitate other standards for children, with the confusion this introduces.

In the face of the evidence, the support of accumulated data seems a doubtful argument to commend a continuance in the use of the DuBois

standard. This historic standard seems to have fewer advantages and more disadvantages than those considered above.

None of these standards may be considered a close approach to true accuracy, and the variation between them in individual cases is not infrequently considerable. It would undoubtedly be good policy in all border-

TABLE XIV

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR MEN

Rate compared	Rate compared with					
	Krogh	Dreyer (observed weight)	Harris-Benedict	Aub-DuBois	Bailey	Mayo
Krogh	0%	−2%	−2%	−7%	−7%	−9%
Dreyer (obs'd weight)	+2%	0%	0%	−5%	−5%	−7%
Harris-Benedict	+2%	0%	0%	−5%	−5%	−7%
Aub-DuBois	+7%	+5%	+5%	0%	0%	−2%
Bailey	+7%	+5%	+5%	0%	0%	−2%
Mayo	+9%	+7%	+7%	+2%	+2%	0%

TABLE XV

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR WOMEN

Rate compared	Rate compared with					
	Krogh	Dreyer (observed weight)	Harris-Benedict	Mayo	Aub-DuBois	Bailey
Krogh	0%	−1%	−2%	−5%	−6%	−6%
Dreyer (obs'd weight)	+1%	0%	−1%	−4%	−5%	−5%
Harris-Benedict	+2%	+1%	0%	−3%	−4%	−4%
Mayo	+5%	+4%	+3%	0%	−1%	−1%
Aub-DuBois	+6%	+5%	−4%	+1%	0%	0%
Bailey	+6%	+5%	+4%	+1%	0%	0%

line or doubtful cases to calculate the basal rate according to at least two of these standards. In these cases correction must be made for the difference in the level of the two standards. This can effectively be done by adding a correction factor obtained from Tables XIV-XVII. Since we must recognize that the standards are imperfect we could in this fashion diminish to a certain degree the likelihood of classifying a high or low normal rate as pathological because of error in the standard. For example, let us consider case 40 of Hobson (male, age 15 years, 11 months, height, 162.7 cm., weight 47.0 kg., caloric consumption at the rate of 1,376 calories for 24 hours).

The basal metabolism by the Harris-Benedict standard is +11 per cent. The rate by the Aub-DuBois standard is −10 per cent, and to make this comparable with the Harris-Benedict we have to add 5 per cent (Table XIV) to correct for the difference of means. The Aub-DuBois comparison rate is −10 per cent +5 per cent = −5 per cent and the Dreyer comparison

TABLE XVI

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR BOYS

Rate compared	Rate compared with				
	Benedict and Talbot	Dreyer (observed weight)	Kestner and Knipping	Mayo	Bailey
Benedict and Talbot	0%	−6%	−7%	−11%	−11%
Dreyer (obs'd weight)	+ 6%	0%	−1%	− 4%	− 5%
Kestner and Knipping	+ 7%	+1%	0%	− 4%	− 5%
Mayo	+11%	+4%	+4%	0%	− 1%
Bailey	+11%	+5%	+5%	+ 1%	0%

TABLE XVII

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR GIRLS

Rate compared	Rate compared with				
	Benedict and Talbot	Dreyer (observed weight)	Kestner and Knipping	Mayo	Bailey
Benedict and Talbot	0%	−8%	−9%	−12%	−16%
Dreyer (obs'd weight)	+ 8%	0%	0%	− 4%	− 9%
Kestner and Knipping	+ 9%	0%	0%	− 3%	− 9%
Mayo	+12%	+4%	+3%	0%	− 4%
Bailey	+16%	+9%	+9%	+ 4%	0%

rate is −8 per cent +0 per cent = −8 per cent. Here, as a rather extreme example, is a difference of from +11 per cent to −8 per cent depending upon the choice of standard even after correction has been made for the differences of mean level. This subject, who, by the Harris-Benedict standard, had a borderline high rate, probably has a normal metabolism.

CONCLUSIONS

1. The empirical test of a basal metabolism standard is its accuracy in the relative prediction of the basal metabolism of normal human beings.

2. For the clinical application of any basal metabolism standard there should be available the mean basal metabolism of a series of normal individuals of the locality, determined under the conditions used for clinical determinations. Such a series should comprise 25 or more individuals.

3. The Dreyer standards based upon observed weight seem on the whole to have the greatest advantages and the least disadvantages. These standards are available in very convenient form in the tables of Stoner (5, 6).

4. In all borderline or doubtful cases the metabolic rate should be calculated by more than one standard, making allowance for the difference in the mean level of the standards. In this procedure one standard based upon the DuBois body surface formula cannot be regarded as a check upon another of the same type.

5. In reporting basal metabolism in the medical literature, the standard used should always be specified.

6. Until such time as there is adequate study of normal human beings to provide definite and statistically significant evidence of the superiority of new standards, this field of investigation is better off without them.

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EFFECT OF VITAMINS A AND D ON RESISTANCE TO INFECTION

By

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THAT laboratory animals in advanced stages of vitamin A deficiency suffer from spontaneous infections is an established fact. Green and Mellanby (1928) found evidence of infection in all but two white rats dying from deficiency of vitamin A. Abscesses at the base of the tongue were the most commonly observed lesion, being present in 72 per cent of such animals examined. Infections of the urinary tract, particularly of the bladder, were very common in Green and Mellanby's series. Mastoid and nasal sinusitis, purulent otitis media, ocular, respiratory and alimentary tract infections occur frequently in vitamin A-starved laboratory animals, apparently because of a lowered resistance to organisms to which the animals were normally exposed. Bacterial and pathological studies of several of these conditions have been made. (Daniels, *et al*, 1923; Werkman, 1923; Goldblatt and Benischek, 1927; Shurly and Turner, 1930; Verder, 1928.)

In contrast to the almost invariable occurrence of infection in animals suffering from deprivation of vitamin A, it is not unusual to encounter the most florid type of rickets, both in the infant and in the experimental animal, unassociated with any apparent bacterial lesion. There is, however, some experimental evidence suggesting lowered resistance to infection in animals deprived of vitamin D.

By decreasing calcium and the antirachitic vitamin in the diet of white rats, Grant (1927) demonstrated a marked increase in their susceptibility to tuberculosis. Eichholz and Kreitman (1928) reported that a much larger proportion of rachitic rats than of normal animals died as the result of a spontaneous paratyphoid epidemic. They also demonstrated greater resistance to injections of pneumococci in white mice fed a rachitic diet supplemented by irradiated ergosterol, in comparison with those receiving the unsupplemented ration.

Robertson (1927) injected rats on a rachitic diet with Gram-negative bacilli isolated from the nose of a normal rat. A smaller percentage of the

animals kept out of doors succumbed to the infection than of those remaining inside. Some increased resistance was also obtained by supplying irradiated ergosterol as the antirachitic agent. For some reason, however, cod liver oil apparently failed to have a similar effect. Hill and Clark (1927) on the other hand, report negative results in an attempt to increase the resistance of rats on an adequate diet, to the inoculation of Type I pneumococci by ultra violet radiation. Ackert and Spindler (1929) were unable to obtain clear-cut evidence that chickens deprived of vitamin D were less resistant to infection by *Ascaris lineata* than were those fed on adequate diets.

We proposed to study further the relation of both vitamin A and vitamin D to infection, endeavoring to obtain evidence of impaired resistance throughout the course of the development of the deficiency. A more accurate demonstration of the influence of these vitamins should be expected before the effects of the depletion became pronounced. It is quite obvious in an experiment of this kind that if animals are infected in the terminal stages of almost any food deficiency, they are apt to succumb readily to the infection.

Experimental

The animals used were four weeks-old white rats reared from mothers on the same adequate stock diet. The young rats of each litter were so distributed that approximately an equal number of litter mates of about the same average weight comprised the test and control groups. They were kept in sterilized wire cages with raised bottoms.

Diets. The vitamin A-free diet (extracted casein 18 per cent, starch 76 per cent, agar 2 per cent and Osborne and Mendel salt mixture 4 per cent) was supplemented each day by 0.5 gram of dry powdered yeast and 0.0015 mg. of irradiated ergosterol in olive oil. In addition to the above diet fed *ad libitum*, the control rats each received 6 drops per diem of a commercial, tested brand of cod liver oil.

The rachitic diet (Steenbock) was made up of ground yellow corn 74 per cent, wheat gluten 20 per cent, calcium carbonate 3 per cent and sodium chloride 1 per cent. The control animals received in addition daily doses of 0.0015 mg. of irradiated ergosterol in olive oil.

The organism. Various pathogenic organisms have been used in similar animal-infection experiments, such as the paratyphoid group, pneumococcus, B. anthracis, B. tuberculosis, B. coli, etc. Since our first attempts were in the direction of producing upper respiratory tract infections, it was thought advisable to obtain an organism naturally found in this location

in the rat. In a previous study, a certain bacillus of the *mucosus capsulatus* group was found to be a common inhabitant in the suppurative lesions of the respiratory tract in vitamin A-deficient rats (Bradford, 1928).

The organism was pathogenic for rats and for other laboratory animals and proved satisfactory for intraperitoneal inoculations in these experiments. Before inoculating each group of animals, the dose was determined by intraperitoneal injections in a series of stock rats of approximately the same age and weight as those to be infected. The infecting dose was taken as that amount of a 1 to 10 dilution of a 24-hour broth culture which just permitted the normal rat to live. It usually ranged between 0.25 cc and 0.4 cc.

Ninety-one rats were used in the experimental series, 46 being put on the vitamin A-free diet, and 45 fed the rachitic ration. Twenty-three rats of the vitamin A group were used as controls and given cod liver oil; likewise 22 of the vitamin D group were given irradiated ergosterol as controls. A group of vitamin A-deficient animals was inoculated along with an equal number of controls respectively, at the end of 4, 6, 8 and 10 weeks upon the experimental diet. The rachitic animals with their controls were divided into three groups which were infected at the end of 4, 6, and 8 weeks respectively.

Post mortem studies. Careful observations of each animal's reaction to the infection and of post mortem findings were made. Those that survived the infecting dose were examined anatomically ten days later. In each animal dying from the infection, acute purulent peritonitis was present. In addition, the lungs, kidneys, spleen and liver showed evidence of acute infection. From the heart's blood and spleen in ten animals cultured, the infecting organism was recovered in pure culture in each case.

In the vitamin A-deficient group, the majority of the test animals infected after 8 to 10 weeks on the diet, showed clinical and anatomical evidences of vitamin A deficiency, such as dry, rough coats, atrophic testes, protruding dried penis and spontaneous infections such as tongue and lung abscesses and purulent sinusitis and otitis. None showed evidence of rickets. In no instance did a control animal show evidence of either vitamin A or D deficiency.

In the rachitic group there was evidence of active rickets in the majority of the animals. In those examined at the end of 6 and 8 weeks, it is interesting to note that spontaneous infections such as characterized the terminal stage of vitamin A-deficient animals were not present. The acute lesions produced by the injected organisms were quite similar. Upon the basis of gross anatomical study, the degree of rickets in the series of sub

groups may be classified as follows: 4 weeks, mild; 6 weeks, moderate; and 8 weeks, severe. The control animals revealed no gross evidence of rickets at autopsy.

Discussion of Results

In Chart 1 graphic representation of the results in the vitamin A-deficient group is given, according to the sub groups infected. In the vitamin

CHART 1.
Vitamin A Deficient Animals.

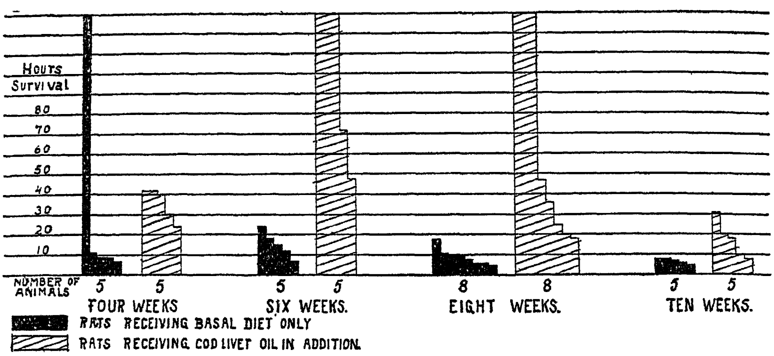
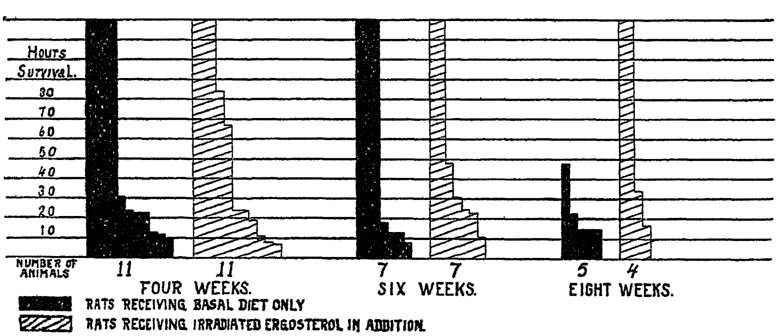


CHART 2.
Vitamin D Deficient Animals.



A-deficient group it is noted that one survived the infection entirely and 22 died. The only survival was in the first sub group infected, which was after four weeks on the experimental diet. The average duration of life after in-

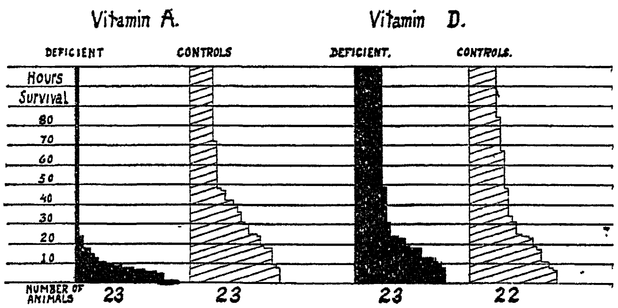
oculation was 10 hours. Of the 23 animals that received the same diet plus cod liver oil (controls) 6 lived and 17 died. The latter survived for an average time of 31 hours.

Chart 2 shows the results obtained among the group of vitamin D-deficient rats. Seven lived and 16 died. Of the controls, 7 lived and 15 died. The rachitic animals survived the inoculation for an average period of 24 hours, while the controls averaged 34 hours.

Chart 3 is a composite graphic comparison of the resistance to infection of the entire groups of deficient animals along with their respective controls.

CHART 3.

Comparative Resistance of Vitamin-deficient Rats to Infection.



As was to be expected from the repeated observations of many infections in animals dying from the lack of vitamin A, the data here presented show a definitely increased susceptibility to infection in the animals fed the vitamin A-free diet, as compared with their controls which received cod liver oil in addition, and also with the vitamin D-deficient group.

Of particular importance, however, is the fact that whereas six to eight weeks on the vitamin A-free diet were required to so deplete the body stores of vitamin A as to result in cessation of growth, yet marked susceptibility to infection was evident as early as the fourth week on the deficient diet. Increased susceptibility to infection is apparently an early manifestation of a dietary low in vitamin A, having marked influence before the physical signs of deficiency are present. The animals in the group inoculated at four weeks were seemingly healthy, active specimens, maintaining a rate of growth not greatly below the controls receiving cod liver oil. This point is of especial clinical significance since classical signs of vitamin A deficiency, such as xerophthalmia, are rarely seen in this country; yet it is

quite probable that relative degrees of vitamin A deficiency exist, predisposing the individual to various types of infection.

The work of Sherman and Burtis (1928) suggests such susceptibility as an early sign of vitamin A depletion. They find further, "that the influence of the level of intake upon the incidence of infection may be pronounced and long continued even when the differences of diet are no greater than may readily occur within the range of ordinary normal or adequate nutrition."

In regard to the vitamin D-deficient animals, the results obtained at the end of 4 and 6 weeks do not show definite evidences of an anti-infective influence of vitamin D. Fewer rachitic animals than controls survived in the group inoculated at the end of eight weeks on the experimental diet, by which time the rickets was well advanced. Undoubtedly an animal or an infant in the terminal stages of rickets may be less resistant to infection or even succumb to it. In fact, it is generally supposed that children suffering from rickets are very susceptible to infections, particularly those of the respiratory tract. A dietary, however, which is so deficient in vitamin D as to lead to the development of active rickets in an infant, very probably is also so deficient in vitamin A as to render the child a more susceptible host to invading organisms. Therapeutically, therefore, it seems important to remedy the vitamin A-deficiency as well as that of vitamin D.

Summary

1. Young white rats were inoculated by intraperitoneal injection of a standard suspension of organisms after 4, 6, 8 and 10 weeks on a vitamin A-free diet. Markedly decreased resistance to such infection, compared with controls receiving cod liver oil, was demonstrated before other signs of vitamin A deficiency appeared.

2. No such susceptibility to similar inoculations was found in young rats on a diet deficient in vitamin D, compared to controls protected by irradiated ergosterol.

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STUDIES IN PROTEIN NUTRITION OF THE CHICK

I. THE INFLUENCE OF DIFFERENT PROTEIN CONCENTRATES ON THE GROWTH OF BABY CHICKS, WHEN FED AS THE SOURCE OF PROTEIN IN VARIOUS SIMPLIFIED DIETS

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FOR the past three years we have been engaged in investigations concerning the comparative value of various commercial protein concentrates as nitrogenous feeds in the poultry ration, particularly in regard to their influence on reproduction. In the course of this work it became evident that to further our knowledge of the dietary requirements for reproduction in the chick, we had to conduct our feeding trials under more carefully controlled conditions. At this time several workers had reported the successful growing of chicks under laboratory conditions, the food requirements being covered by synthetic diets. We decided to avail ourselves of this information and to determine first the comparative growth value of these protein supplements when fed as the sole source of protein in an otherwise synthetic diet. The present paper presents some of the difficulties which we have encountered, showing that our present concept of synthetic diets for chicks is quite inadequate. The protein feeding stuffs studied were buttermilk powder, fish meal, cod-liver meal and two abattoir by-products, meat meal and tankage.

Experimental

Our objective was to procure a synthetic basal diet, which, while satisfying the vitamin requirements, etc. of the chick, contained the minimum amount of protein and to supplement this diet with equal amounts of crude protein from the different protein supplements. A review of the literature revealed that our greatest difficulty in using a synthetic basal diet was to prevent the incidence of leg-weakness. Hart, Halpin and Steenbock (1920) found that lack of a suitable roughage in the diet was the major cause of leg-weakness and that normal chicks could be grown on a

synthetic diet composed of butter fat 15; dried yeast 15; salts 5; casein 18; dextrin 37 and paper 10; the latter being ground into the diet. Later, Hart, Steenbock and Lepkovsky (1924) reported that more uniform growth could be obtained by replacing the butter fat with 5 per cent of an ether extract of saponified cod-liver oil. In our first series of experiments, a modification of this synthetic diet was used as our basal diet.

First Series

The total fat content of these protein supplements is not only extremely variable (see Table I) but the vitamin A and vitamin D contents are

Table I

Percentage Composition of Protein Supplements

	Meat Meal	Tankage	Fish Meal	Cod-Liver Meal	Buttermilk Powder
Moisture	3.84	5.89	5.90	3.4	7.62
Crude protein ($N \times 6.25$)	55.90	56.70	75.77	41.9	34.80
Ether extract	14.58	10.91	3.86	44.1	5.31
Crude fibre	1.22	1.79	0.23	0.3	—
Lactose	—	—	—	—	37.91
Lactic acid	—	—	—	—	5.82
Total ash	19.51	20.28	17.63	2.83	8.47
Silica	0.44	0.68	1.90	0.05	.20
P_2O_5	6.85	6.72	5.55	0.64	1.48
CaO	8.55	8.74	5.97	0.15	1.23
MgO	0.59	0.61	0.88	0.09	.22
Na_2O	1.02	1.55	1.81	0.67	2.25
K_2O	0.44	0.88	0.48	0.25	.41
Fe_2O_3	0.04	0.15	0.016	0.009	0.007
Cu(Mg/Kg)	5.4	7.2	4.2	13.35	3.8

also quite variable in the different materials. As we were particularly concerned with a comparison of the protein of these materials, we have freed them from the variable amount of fat which they contain by extracting with ether in a large copper Soxhlet extractor (Bryant, 1929). This procedure was adopted in all the experiments described. At the time these experiments were begun the inorganic analysis (Table I) had not been completed. The amount of ether extracted meal, required to give 18 per cent crude protein, added to the diet the largest amount of ash, 8.9, per cent so that it was decided to bring the ash content of all the diets to this level using the ash of fish meal. We felt, in view of the high value which Orr (1925) has placed on the inorganic constituents of fish meal, that

any difference in the composition of the ash of the different protein supplements which might be a limiting factor in growth would be overcome by this procedure. The results of the ash analysis when completed showed that this assumption was justified.

The following "basal" synthetic diet was supplemented by 18 per cent crude protein from each of the protein concentrates.

		Per cent
Yeast extract		10
Butter oil		10
Cod liver oil (saponified)		2
Paper pulp		10
Fish meal ash	to	8.9 total ash
Dextrin	to	100

Ultra-violet light—10 min. twice weekly.

Our endeavour was to obtain a source of vitamin B which would not necessitate the addition to the diets of large amounts of extraneous nitrogenous compounds. An alcoholic extract of dried brewer's yeast, prepared according to Hartwell (1922), was used. The saponification of the cod-liver oil and the ether extraction of the unsaponifiable residue was carried out according to Steenbock, Jones and Hart (1923). A high grade medicinal cod-liver oil was used and an amount of the unsaponifiable residue in ether equivalent to 2 per cent of the original oil was added to the diets and the ether evaporated off. A high grade paper pulp was dried and ground in a Wiley mill and then 10 per cent of the ground paper was reground into the diets.

The experiment was commenced with day-old barred rock chicks, thirty in each group, housed in Carrick Brooders (1925) in the laboratory. At the end of ten days the number in each group was reduced to twenty by weeding out those chicks showing evidence of constitutional weakness. Distilled water was before the birds at all times and the diets were prepared in amounts to last approximately one week. Post mortem examination was made on all birds that died and hemoglobin determinations (Newcomer) were made on all the birds which survived the six weeks duration of the experiment.

NOTES

GROUP I. *Buttermilk Powder:* Chicks hatched May 21; feeding commenced May 24. Throughout the experiment these were strong healthy chicks. Their feces were extremely watery and by June 1 they were extremely dirty. On June 2, several of the heavier chicks showed deformity of the toes, the digits of the foot becoming crooked. On June 5, the ultra violet irradiation was increased to 20 min. without effect on the toe deformity. On June 9, the biggest chicks developed leg-weakness, walking with a stilted gait, their legs bending at the hock joint. The joints of the metatarsus and

the digits became puffy. By June 11, three birds were paralysed in the legs and wings—the joints being badly swollen and having a bluish color. By June 18 the yeast extract was increased to 15 per cent and four birds were receiving 40 mg. yeast vitamin B concentrate (Harris) per day, in a gelatin capsule. The birds were only prevented from eating heartily by complete paralysis. On July 2 the experiment was concluded with 14 birds surviving, all more or less suffering from leg-weakness and appearing to be anemic. The average hemoglobin content of the blood was 8.5 gms. per cent with individual variations from 7.6 to 9.4 gms. per cent. The blood volume was low.

GROUP II. *Meat Meal:* Chicks hatched May 22; feeding commenced May 26. Little or no growth was made by this group. By May 31 the chicks showed an apparent loss of vigor, preferring to remain under the hover. On June 13 there were only nine birds left and these had made little or no growth. On June 29 the experiment was discontinued with one bird surviving. Certain birds in this group showing signs of failing were also given 40 mg. per day yeast vitamin B concentrate without effect. In general, the post mortems showed the liver to be badly congested, the kidneys hypertrophied with many small hemorrhages and the gall bladder small, transparent and gelatinous. The ureters and cloaca were almost plugged with urates. One outstanding pathological condition noted in many cases was that the lining membrane of the gizzard was eroded and exfoliated medially.

GROUP III. *Tankage:* Chicks hatched May 22; feeding commenced May 26. The results with this group were very much the same as with Group II. The same dull, unthrifty appearance with little or no growth. Cannibalism, toe and feather picking, was practiced to a large degree. The last chick died on June 27. In general the post mortem conditions were as in Group II, but the eroded and exfoliated condition of the lining membrane of the gizzard was more severe and more prevalent. In some cases the lining membrane of the gizzard was practically destroyed; in others it was darkened, shrunken and appeared as if slightly burned, suggesting that something toxic was present in meat meal and particularly tankage.

GROUP IV. *Fish Meal:* Chicks hatched May 23; feeding commenced May 26. During the first two weeks these chicks were the best of the entire experiment. Around June 20 a general dullness manifested itself and the mortality was great, all the chicks being very anemic. What appeared to be big healthy chicks died and were found to be extremely anemic, the blood volume being also extremely low. The experiment was concluded on June 30, chloroforming the last remaining bird. From June 13, on first signs of failing, several chicks received 40 mg. yeast vitamin B concentrate per day with no apparent effect. The outstanding observation in the post mortems was the extreme general anemia. Hemoglobin determinations on a number of birds were made, the highest being 7.7 gms. per cent and the average 6.3 gms. per cent. A 1 per cent $CuSO_4$ solution was administered to a number of birds by pipette, without any beneficial result.

GROUP V. *Cod Liver Meal:* Chicks hatched May 23; feeding commenced May 26. Up to June 13 this group did very well but from this date the birds developed chronic diarrhoea. On June 23, 5 per cent charcoal was added to the diet and as a result the feces were greatly improved. There were fewer losses in this group than in any other, excepting Group I. On July 3 this experiment was concluded with 10 chicks surviving. At this time, while they were growing fairly well, they did not seem to be feathering properly and had rather a dull grey color. Hemoglobin determinations were made on the surviving birds, the average result being 10.71 gms. per cent which was higher than in any other group. The chicks seemed to have a remarkably great blood volume compared to the other groups. The mortality was confined to chicks showing extreme ante-mortem diarrhoea.

Chart 1 shows the average growth in grams obtained with each group. The weekly mortality is also given. It will be seen that the growth on none of these diets was satisfactory. While fair growth was made by group I, the incidence of extreme leg-weakness showed this diet to be far from satisfactory. The complete failure of the meat meal and tankage groups was very surprising.

Palmer and Kennedy (1928) have reported that vitamin G (B_2) is only very slightly soluble in water or 90 per cent ethyl alcohol. It seemed then not improbable that the yeast extract which we used may have contained very little of the growth factor present in the yeast and this might account for the unsatisfactory growth in this experiment. We then repeated group I replacing the 10 per cent yeast extract by an equivalent amount of the untreated brewer's yeast, *i.e.*, Group VI. To determine to what extent the yeast protein supplemented the protein of this diet we also put one group on the same diet as Group VI except that the buttermilk powder was replaced by an equivalent amount of crude protein from pure edible

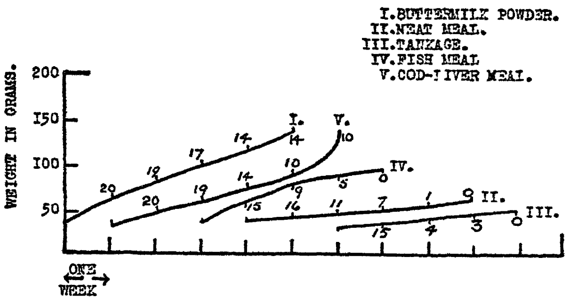

Chart 1.—Showing the growth with the different proteins supplementing a basal diet composed of yeast extract 10%; butter oil 10%; cod-liver oil (sap.) 2%; paper pulp 10%; fish meal ash to 8.9%; total ash, and dextrin to 100.

Note. In these, and in all other growth curves recorded in this paper, the arabic figures on each curve give the number of chicks living at each particular time.

gelatin, *i.e.*, Group VII. A third group was fed the same diet as Group VI except that in place of brewer's yeast, filtered tomato juice, concentrated in vacuo at 140°F. was used as the source of vitamin B an arbitrary amount (the equivalent of 25 cc. of the original unconcentrated juice per bird per day), being given, *i.e.*, Group VIII. The remainder of the diet and the conditions under which this experiment were conducted, as regards the number of chicks, etc., were the same as has been described.

NOTES

GROUP VI. *Buttermilk Powder, Brewer's Yeast:* This group made better growth but developed leg-weakness to a greater extent and in even greater severity than did Group I. On the first signs of approaching leg-weakness, as evidenced by the crooked toes, the amount of yeast was increased to 15 per cent and a few days later to 20 per cent, replacing an equivalent amount of dextrin, without delaying the onset of the leg paralysis. The three mortalities were chicks which were completely paralysed, death being due to the inability of the birds to move about and feed. The average hemoglobin content of the blood of the surviving birds was 9.23 gms. per cent with variations from 8.5 to 13.6 gms. per cent. It was difficult to obtain blood from the brachial artery as the blood volume was extremely low. Ash determinations on the dry, fat-free, femurs and tibias and blood

serum calcium and phosphorus determinations were made on six chicks from this group which were suffering from extreme leg-weakness with the following results:

Sample No.	Percentage Ash Femur	Percentage Ash Tibia	Calcium* (Mg. per 100 cc.)	Phosphorus** (Mg. per 100 cc.)
1.	52.84	52.99	14.9	6.10
2.	55.98	53.58	14.4	6.37
3.	62.56	58.24	13.8	6.62
4.	56.23	53.53	14.4	5.83
5.	62.25	51.05	13.3	5.88
6.	58.68	—	14.9	6.50

* Determined by the Clark-Collip modification of the Kramer-Tisdall method (1925).

** Determined by the method of Fiske and Subbarow (1925).

GROUP VII. *Gelatin, Brewer's Yeast:* Chicks hatched May 24. Feeding commenced May 26. By June 8 there were only 11 chicks left in the group and these had not, in some cases, even maintained their original weights. Those that died, apparently from starvation, had practically completely absorbed the yolk sac. The mortality and loss in weight continued until June 13 when the amount of yeast was increased to 15 per cent. The improvement was very slight; while some made a little gain in weight, others lost weight and died. The extreme cannibalism, toe, head and plumage picking, which had been outstanding in the group still continued. The experiment was concluded on July 3, with two chicks living. Hartwell (1926) found that yeast extract supplemented gelatin in a synthetic diet for rats, and Osborne and Mendel (1919) were successful in growing rats on a diet in which yeast furnished the sole source of protein. Funk, Lyle, and McCaskey (1916), however, assert that "a large part of the yeast nitrogen apparently has no food value, it being badly assimilated by rats."

GROUP VIII. *Buttermilk, Powder—Tomato Juice:* Chicks hatched May 24. Feeding commenced May 26. At the outset it was found impossible to feed the tomato juice by placing it before the chicks as they would not consume very much and when allowed access to water they drank as if they had been thirsty for some time. The concentrated juice was then mixed into the diet. On May 29, 5 chicks died, all having greatly enlarged abdomens and showing a post-mortem condition of general oedema. A change was made, incorporating the original unconcentrated tomato juice into the diet in an amount to give about 20 cc. per bird per day. From this time the birds made fairly good gains in weight, but by June 27 developed leg-weakness just as did Groups I and VI. On July 3 the experiment was discontinued with one bird living. The mortality was either the result of general oedema or extreme leg-weakness.

In Chart 2 is shown the average growth in grams and the weekly mortality of these three groups compared with Group I. The growth in Group I with yeast extract was not as good as the growth with Group VI with untreated medicinal brewer's yeast. It seemed probable that the results obtained with fish meal, meat meal, tankage and cod-liver meal may have been similarly affected by a lack of vitamin G (B_2) in the yeast extract used. It was, therefore, decided to repeat the experiments with these four protein supplements using the unextracted dried brewer's yeast.

SECOND SERIES

In repeating the experiments with Groups II to V inclusive, the following changes were made in the diets. Each group received 15 per cent of

dried brewer's yeast. The ether extract of the unsaponifiable residue of cod-liver oil was dispensed with and all groups received 5 per cent medicinal cod-liver oil. The experiment commenced on July 31 with selected, day-old Barred Rock chicks, thirty in each group. Each group was further divided at feeding time into three lots of ten chicks; an arbitrary change was made in the amount of crude protein from the protein concentrate and in the fibre content of the diets in each lot, as follows:

Lot I	Lot II	Lot III
18% Crude protein	12% Crude protein	18% Crude protein
10% Paper pulp	10% Paper pulp	5% Paper pulp

The composition of the remainder of the diet, except for the change in the yeast and cod-liver oil, was as in the first series of experiments.

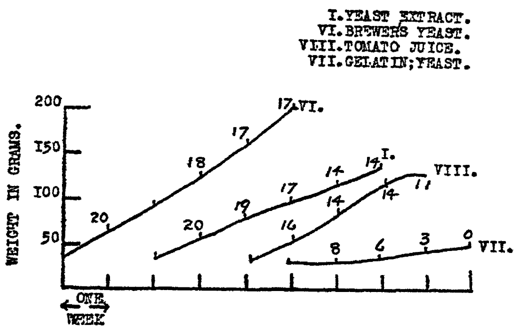

Chart 2.—Showing the comparative growth with buttermilk powder and different sources of vitamin B, and the growth with gelatin and brewer's yeast.

NOTES

Group IX. *Tankage:* The growth of the chicks in this group showed very little improvement from that obtained with Group III, particularly in the case of lots I and II. There was a slight improvement in lot III where the fibre content of the diet was halved. Cannibalism was just as bad as in Group II and the post mortem conditions were again characteristic of starvation and general emaciation.

Group X. *Meat Meal:* Extremely poor results were obtained with this group, there being little or no growth with all three lots and the mortality was just as bad as in Group II. The post mortem conditions were, as before, characteristic of complete starvation. The change in the amount of meat meal and fibre in the diet had little or no effect. It was characteristic of this group that they weakened very quickly, showed little or no interest in their food and then died.

Group XI. *Fish Meal:* The results obtained with this group were much the same as with Group IV. For a time the chicks did extremely well, but were always pale and anemic looking. From the fourth week we continued to lose several chicks each day. A big, healthy chick would appear quite normal and then, within a few hours, become suddenly weak and unsteady on its legs and in a short time fall over, stiffen out and die. A typical post mortem examination of such a mortality revealed the viscera to be colorless, the kidneys, lungs, and liver being very pale and the heart seemingly bloodless. Numerous pin-point hemorrhages in the kidneys and an excess of water in the ureters and cloaca were noticed. In a few cases there were great blotches of hemorrhages all along

the left side and left leg, particularly about the femur. This group received $CuSO_4$ daily in their drinking water and in many cases daily capsules containing 40 mg. yeast vitamin B concentrate (Harris) were given. In general the chicks seemed to make excellent growth to a certain point and then die in a very anemic condition. There was little or no difference in the different lots in this group.

GROUP XII. *Cod Liver Meal:* The results obtained with this group were disappointing and in direct variance with those obtained with Group V. The growth in all lots was poor and the mortality particularly heavy. There was little or no difference in the three lots.

In Chart 3 is shown the average growth in grams and the weekly mortality of these three groups. The failure of these protein supplements in this type of nutritional experiment is hard to explain. Since fair growth was obtained with buttermilk powder, it may be that the other protein

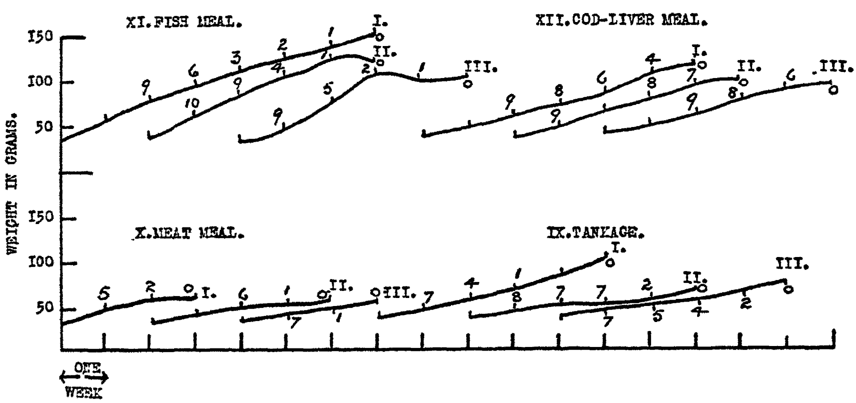

CHART 3.—Showing the effect of varying the amount of protein and fibre in the different diets. Each group was divided into three lots, the same basal diet being supplemented with; I. 18% protein and 10% paper; II. 12% protein and 10% paper; 18% protein and 5% paper.

concentrates, particularly meat meal and tankage, are deficient in one or more essential amino acids. There was evidence, however, that in the case of tankage a change in the physical condition of the diet by reducing the amount of fibre was slightly beneficial. Palatability and digestibility may have been to some extent a limiting factor. Waterman and Jones (1921–1922) have described a method for determining the digestibility of proteins by pepsin and trypsin *in vitro*, the results of which correlate with the results of growth experiments with rats. We have applied this method to these protein concentrates; the results, however, (Table II) did not parallel the results of the growth experiments with chicks and are of little value in explaining the nutritional behaviour of these proteins.

We consistently observed that the chicks in the tankage and meat meal groups seemed very constipated, while the chicks receiving buttermilk

powder seemed extremely laxative. It was thought that if the tankage and meat meal diets could be made more laxative better growth might be obtained. Replacing the dextrin of the meat meal and tankage diets by an amount of lactose and lactic acid, equivalent to that in the buttermilk powder diet, while having a pronounced laxative effect on the chicks, gave no better growth and the mortality was just as great as ever.

Third Series

It would seem that since leg-weakness developed in the buttermilk powder group, where otherwise growth was fairly satisfactory, the basal

TABLE II

COMPARATIVE DIGESTIBILITY OF PROTEIN CONCENTRATES AS DETERMINED IN VITRO BY THE METHOD OF WATERMAN AND JONES

	% Total N.	% Total amino N.	% Digested amino N. on basis of Total N.	% Digested N. on basis of Total amino N.	% of Total N. as amino N.
Buttermilk powder.	4.53	3.46	31.61	41.39	76.36
Tankage.........	11.91	7.68	14.82	22.98	64.49
Meat meal.......	10.32	6.56	26.31	41.37	63.61
Fish meal........	12.89	7.97	38.40	52.09	61.83
Cod-liver meal....	10.79	6.81	12.69	20.09	63.03

diet which we have been using was not adequate. In view of this fact we hesitated to draw any conclusions from the results with the other protein supplements. That there is a definite relationship between the protein and vitamin B of the diet has been suggested by the experiments of Plimmer *et. al.* (1927) with chicks, and by those of Hartwell (1928) with rats. By varying the ratio of buttermilk powder to brewer's yeast in the diet used with Group VI, an attempt was made to prevent the occurrence of leg-weakness. As only a few pens were available at the time, only a limited number of arbitrary levels of protein to yeast were tried, as follows:

GROUP XIII.	Ratio Protein:Yeast
Lot I, 15% protein from buttermilk powder:15% yeast	1 : 1
Lot II, 12.5% protein from buttermilk powder:15% yeast	1 : 1.3
Lot III, 10.0% protein from buttermilk powder:15% yeast	1 : 1.5
Lot IV, 10.0% protein from buttermilk powder:10% yeast	1 : 1
Lot V, 7.5% protein from buttermilk powder:15% yeast	1 : 2
Lot VI, 7.5% protein from buttermilk powder:10% yeast	1 : 1.3

The basal synthetic diet was the same as previously described with the buttermilk powder Groups I and VI, except that the amount of medicinal cod-liver oil was increased to 5 per cent. The lactose and lactic acid content was kept the same in all the diets by adding varying amounts to the basal diet depending upon the amount of buttermilk powder. The experiment was commenced with fifteen one-day old barred rock chicks in each lot and was conducted under the conditions already described.

Chart 4 shows the average growth in grams of each of the lots for the six weeks duration of the experiment. It was found that 15 per cent protein from buttermilk powder was the minimum from this particular source which would give reasonable growth comparable with Group VI receiving 18 per cent crude protein from buttermilk powder. Less than 15 per cent protein from buttermilk powder gave distinctly subnormal growth. With

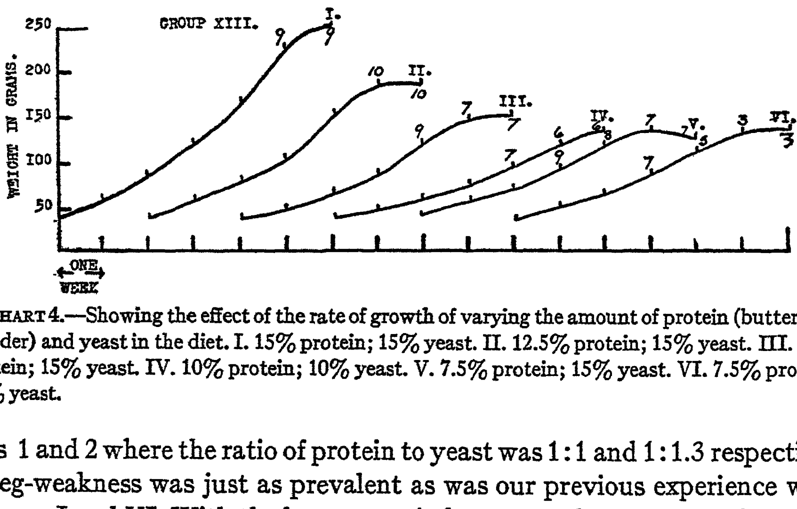

CHART 4.—Showing the effect of the rate of growth of varying the amount of protein (buttermilk powder) and yeast in the diet. I. 15% protein; 15% yeast. II. 12.5% protein; 15% yeast. III. 10% protein; 15% yeast. IV. 10% protein; 10% yeast. V. 7.5% protein; 15% yeast. VI. 7.5% protein; 10% yeast.

lots 1 and 2 where the ratio of protein to yeast was 1:1 and 1:1.3 respectively leg-weakness was just as prevalent as was our previous experience with Groups I and VI. With the lower protein lots even where the protein:yeast ratio was 1:2 (lot 5) leg-weakness still developed although much less severely than in the higher protein groups, lot 1 and 2. This was consistent with our previous finding in Groups I and VI that individual birds making the greatest gain in weight developed severe leg-weakness before the slower growing individuals.

Hogan and Shrewsbury (1930) have reported that good growth and freedom from leg-weakness was procured only when their simplified casein diet was fortified by 40 per cent of yeast. Jack (1926) likewise found that in using synthetic diets with chicks, leg-weakness could be prevented only by increasing the amount of brewer's yeast away beyond what might reasonably be considered the optimum vitamin B requirements of the chicks.

FOURTH SERIES

So far, it had not been found possible to grow normal chicks with a diet in which, excepting for the brewer's yeast, the protein was entirely of animal origin. Plimmer, *et. al.* (1927) have reared chicks successfully on a diet composed of fish meal 15; dried yeast, 20; cod-liver oil, 1; and white rice, 90 parts. This diet is not essentially different from those used by us, *e.g.*, Group XI, except that instead of white rice we have used dextrin, as a source of carbohydrate and ground paper pulp as roughage. When the fish meal was increased to 20 parts, the chicks developed leg-weakness which the authors suggested was due to the kind of protein, as previously they had reared first-class birds using as high a proportion of milk proteins. (See Plimmer and Rosedale (1922.)) In these experiments using caseinogen and dried whey, however, the remainder of the diet was not the same as used in 1927, *e.g.*, oatmeal was used instead of white rice. Believing that our failure to grow normal chicks was associated with the composition of the basal synthetic diet, either in the use of dried yeast, paper or dextrin, we next repeated these feeding trials with the different fat-free protein concentrates, supplementing the following basal diet:

Marmite	15%
Cod-liver oil	3%
Iodized salt*	1%
Bone meal** to	4.36% total ash
White rice to	100.

* Early in the experiment the use of the iodized salt was discontinued. We were not aware that the NaCl content of the marmite was already so great. A number of the baby chicks developed oedema which, however, ceased as soon as the extra salt was removed from the diet.

** Each of the protein supplements was added to the different diets in an amount to supply the equivalent of 10 per cent crude protein (N×6.25). The ash content of the diets was then found to vary within narrow limits. The total ash content of all the diets was brought to the same level 4.36 per cent by adding bone ash. The largest amount added was in the buttermilk powder diet and this was only 0.33 per cent.

It was only necessary to vary the amount of white rice in the different diets within narrow limits, *i.e.*, 65.8 to 67.3 per cent except in the buttermilk powder diet Group XV. The protein content of the buttermilk powder was much less than that of the other protein supplements, so a larger amount of buttermilk powder was required to add 10 per cent protein to the diet and therefore, the amount of white rice was proportionately reduced to 49.3 per cent. The use of tankage as a protein supplement was discontinued. To obtain some idea as to whether the protein of the marmite and white rice was of good quality, Group XVIII was fed

the basal diet with edible gelatin as the source of protein. Again as a check on the marmite used, two groups were given 10 per cent of protein from casein with the same basal diet as before, except that group XIX received 15 per cent marmite while group XX received 15 per cent dried brewer's yeast. With the gelatin and the two casein diets 4 per cent mineral mixture (See Hart *et. al.* (1920)) was added.

The experiment was commenced with over thirty barred rock chicks, one day old, in each group, housed under the conditions already described. At the end of the first week the numbers were reduced to 25 chicks in each group, discarding those showing any evidence of constitutional weakness. Each group received daily ultra-violet irradiation at a height of 24 inches for 10 minutes.

NOTES

GROUP XIV. *Buttermilk Powder:* The growth of these chicks was excellent but throughout the experiment they always had a peculiar appearance. The feathers were moist and ruffled, giving the chicks a wet appearance in the region of the breast and head. The six chicks which died during the first three weeks were all suffering from oedema, the pericardium being filled with a straw-yellow, viscous fluid.

GROUP XV. *Meat Meal:* While the growth of these chicks throughout the experiment was good, the mortality was heavy. Four chicks were lost as the result of hemorrhage on inserting the identification wing bands in the web of the wing; the chicks bleeding slowly for from 12 to 48 hours before death.

GROUP XVI. *Fish Meal:* Twenty chicks were lost in this group during the first four weeks. The post mortems on 19 of these chicks showed a hemorrhagic condition, the hemorrhage spots in a few cases being peritoneal clots but more frequently small blotches along the muscles of the femur and sternum. These chicks looked strong, healthy and vigorous until a few hours before death. The death of eight chicks in this group in the fourth week was the result of inserting the identification wing bands in the web of the wing which caused a fatal hemorrhage, bleeding continuing for from 12 to 48 hours before death.

GROUP XVII. *Cod Liver Meal:* At no time during the eight weeks duration of the experiment did the chicks in this group attain normal weight. The chicks always had an unthrifty appearance and evident lack of vigor but only 5 chicks were lost, these showing no one particular post mortem condition.

GROUP XVIII. *Gelatin:* The chicks in this group were always distinctly abnormal, their growth was slow, and their appearance sickly, the plumage being ruffled. The mortality was heavy and regular in frequency. No typical post mortem condition was observed.

GROUP XIX. *Casein, Marmite:* From the beginning these chicks grew excellently. They appeared normal in every respect at the conclusion of the eight weeks. No mortality occurred after the first week when one chick was lost.

GROUP XX. *Casein, Brewer's Yeast:* These chicks grew well for the first two weeks. During the third week, rapid growth continued with very little mortality, but the chicks exhibited a peculiar nervousness when the pen was approached. Six chicks died in the fourth and fifth week, all showing the same ante-mortem condition. The birds lay prone on one side with the legs outstretched and the head twisted back in a typical polyneuritic manner. Death followed in 12 to 24 hours. No gross pathological condition was observed on post mortem examination. After the fifth week, there were no further losses, the chicks continuing to grow normally. The ratio of units of food required to produce a unit of gain with the different groups was as follows:

Group	XIV.	Buttermilk powder	2.4:1
"	XV.	Meat meal	2.8:1
"	XVI.	Fish meal	2.4:1
"	XVII.	Cod-liver meal	3.5:1
"	XVIII.	Gelatin	3.8:1
"	XIX.	Casein—marmite	2.6:1
"	XX.	Casein—brewer's yeast	2.3:1

In Charts 5 and 6 are recorded the mortality and the average growth of these groups. It will be noted that only with cod-liver meal as the protein

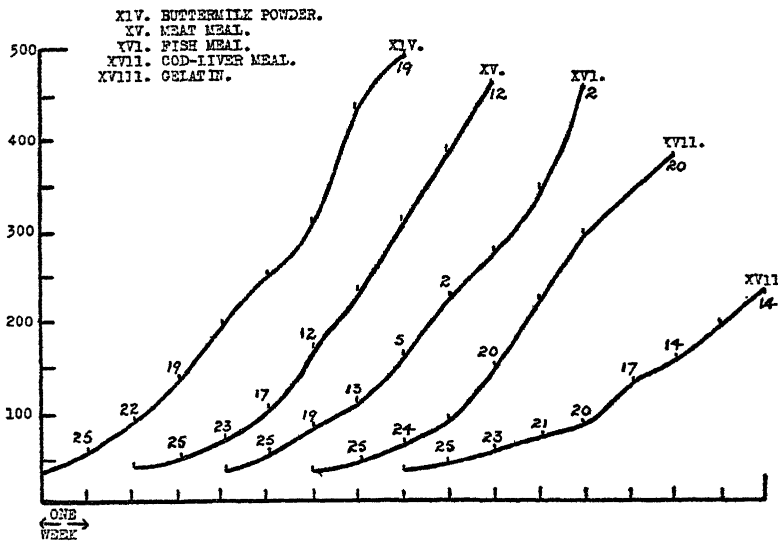

CHART 5.—Showing the growth with the different proteins when supplementing a basal diet composed of marmite 15%; cod-liver oil 3%; bone ash to 4.36% total ash, and white rice to 100.

supplement was growth subnormal. From the fifth week only two birds remained in the fish meal group; their growth, however, continued normally. Poor growth was obtained when the basal ration was supplemented by gelatin. The growth with the gelatin diet, however, was such, that the protein of marmite and the small amount of protein (2.6 per cent) from the white rice may have had a marked supplementary effect on the comparative growth, with the different protein concentrates. The heavy and characteristic mortality in Group XX as compared with Group XIX would suggest that 15 per cent of the dried brewer's yeast used in this and in previous experiments, was close to the minimum required to maintain normal nutrition. It would appear that the amount of vitamin G (B_2) was adequate, as good growth was attained by the surviving birds. The ante-mortem

symptoms exhibited by the chicks suggested that vitamin B_1 was deficient in the particular yeast used.

Fifth Series

It occurred to us that, since replacing the dried brewer's yeast in the diet by an equivalent amount of marmite reduced the high mortality to nil, the limiting factor in the synthetic basal diet used in our earlier experiments might still be the brewer's yeast. We desired to find some substitute for the large amount of white rice used in these diets as evidently the small

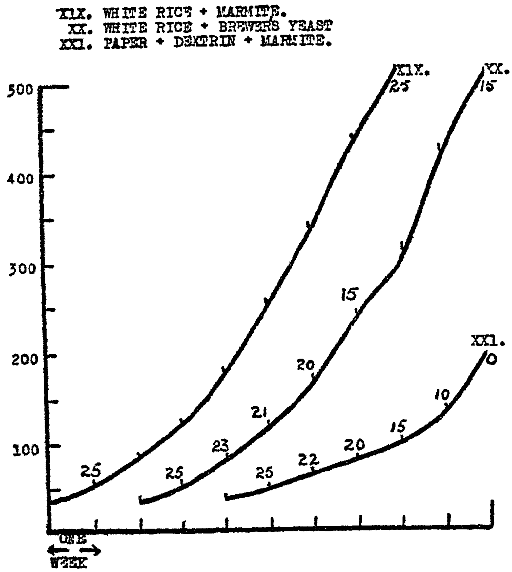

Chart 6.—Showing the comparative growth with casein as the source of protein in different simplified diets.

amount of protein in the rice was of good biological value. Our next attempt to obtain normal growth with a more strictly simplified diet was made by supplementing the following basal diet with an amount of each of the different fat-free protein concentrates sufficient to add 12.5 per cent crude protein to the diet, thus keeping the total protein the same as with the fourth series of experiments.

Marmite	15%
Cod-liver oil	3%
Paper pulp	3%
Bone ash to	4.36% of total ash.
Dextrin to	100.

In Chart 7 are shown the results in growth and mortality of this feeding trial. While the growth with fish meal and cod-liver meal was for some unexplained reason an improvement on the growth obtained with buttermilk powder or meat meal, in all cases the results were a failure, growth being

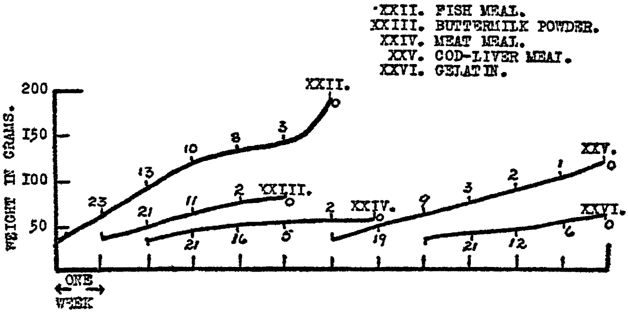

CHART 7.—Showing the growth with the different proteins when supplementing a basal diet composed of marmite 15%; cod-liver oil 3%; paper pulp 3%; bone ash to 4.36% total ash, and dextrin to 100.

decidedly sub-normal and no chick surviving the sixth week of the experiment. In Chart 8 are shown the results of an attempt to improve this diet by varying the amount of fibre. Three per cent of fibre from paper pulp gave the best results, but in all the groups the growth was again very poor and the mortality 100 per cent in six weeks.

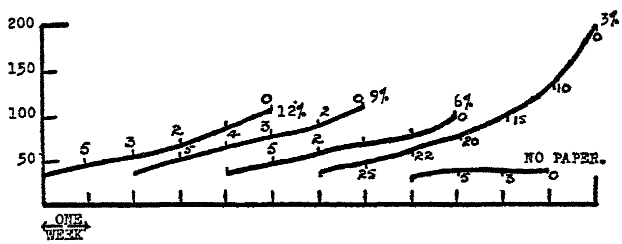

CHART 8.—Showing the effect on growth of varying the amount of fibre (paper pulp) in a diet composed of 12.6% protein from casein; marmite 15%; cod-liver oil 3%; bone ash to 4.36% total ash, and dextrin to 100.

That the occurrence of leg-weakness among chicks reared in confinement on a synthetic diet could be prevented by increasing the amount of dried brewer's yeast to 40 per cent of the diet, has already been discussed. We have found, however, that keeping the amount of brewer's yeast constant at 15 per cent and changing the composition of the remainder of the basal synthetic diet, *i.e.*, replacing the paper and dextrin by white rice made the diet entirely adequate for normal nutrition. It occurred to us that possibly

the small amount of vegetable protein in the white rice was the cause of marked improvement and that it was not possible to grow normal chicks on a diet in which the protein was entirely of animal origin. It seemed possible that, as the large amount of yeast required to prevent leg-weakness was greatly in excess of the vitamin B requirements of the chick, some factor other than the vitamin B complex of the yeast, *i.e.*, the yeast protein, was responsible for the improved nutrition. In Chart 9 are recorded the results of a preliminary experiment to determine whether normal growth could be obtained by increasing the amount of yeast using 5 per cent of the residue of brewer's yeast which had been thoroughly extracted with 90 per cent ethyl alcohol. Group XXXI was given the same basal diet but with an equal amount of crude protein from wheat gluten to that added in

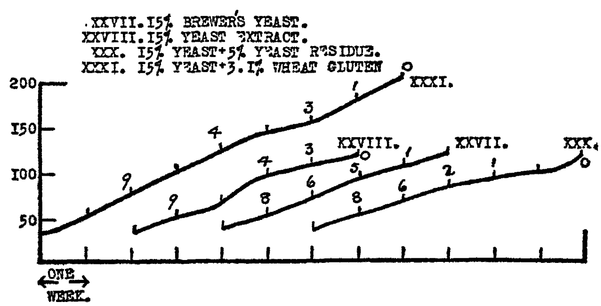

CHART 9.—Showing the effect on growth of different additions to a diet composed of 18% protein (buttermilk powder); 10% paper pulp; 15% cod-liver oil; fish meal ash to 8.9% total ash, and dextrin to 100.

the 5 per cent yeast residue. There was little or no difference in the results obtained with brewer's yeast, 90 per cent ethyl alcohol extract of brewer's yeast or with brewer's yeast plus 5 per cent alcohol extracted yeast residue. The addition of a small amount of wheat gluten did considerably improve the growth. The results, however, were uniformally poor, the total protein of the diets being probably too high.

DISCUSSION

Our experiments using synthetic diets have not been so satisfactory as we had hoped. We have encountered many difficulties which show definitely that the nutritional requirements of the chick cannot be adequately met by the synthetic diets being used successfully with rats. Our original objective to study the comparative value in avian nutrition of different proteins when fed as the sole source of protein in an otherwise synthetic diet, has not been attained.

It has been found necessary, if normal growth is to be obtained, to include in the basal diet food stuffs such as marmite and white rice or brewer's yeast and white rice, which add to the diet a considerable amount of vegetable protein. A diet composed of casein 12.2 per cent, marmite 15 per cent; cod-liver oil 3 per cent; salt mixture 4.4 per cent and white rice 61.8 per cent *i.e.*, Group XIX, grew perfectly normal chicks with no mortalities after the 1st week of growth but when 3 per cent paper pulp and dextrin was ground into the diet in place of the white rice and the total protein of the diet was kept the same by increasing the casein to 15.8 per cent *i.e.*, Group XXI, more or less complete failure resulted, none of the 25 chicks surviving the sixth week of the experiment. The poor growth could not be attributed to the fibre content of the diet as varying the amount of paper pulp (see Chart 8) did not result in any improvement. The difference between success and failure with Group XIX and Group XX can be due only to some substance present in the white rice. While Susuki (1927) has shown the protein of white rice to be of good biological value it would be very surprising that the small amount of rice protein 2.6 per cent added to these diets should have such a pronounced supplementary effect on casein and more particularly on the protein of buttermilk powder, Group XIV, Chart 5. However, the improved growth resulting from the addition of a small amount of wheat gluten, 3.1 per cent to a synthetic diet (Group XXXI, Chart 9) would suggest that the effect of the inclusion of white rice in the diet might be due to the rice protein. Comparing the results with Group XXVI (Chart 8) and Group XVIII (Chart 5) there is no doubt that 2.6 per cent protein from white rice had a marked supplementary effect on the protein of marmite and gelatin. However, it can only be concluded from these experiments that the addition of white rice to these synthetic diets, proven to contain adequate amounts of the known vitamins essential for growth in the chick, had a pronounced effect in improving the growth and reducing the mortality, which cannot be explained from our present knowledge of the nutritional requirements of the fowl. That the effect is due to the rice protein, and that some protein of vegetable origin, or some unknown factor associated with vegetable material, is necessary for normal nutrition of the chick is suggested, but has still to be definitely proven by further experiments.

Equal amounts of crude protein from buttermilk powder, fish meal or meat meal had practically the same effect on growth when supplementing a basal diet composed of marmite 15 per cent; cod-liver oil 3 per cent; bone ash to 4.36 per cent total ash and white rice to 100. However, supplementing the same basal diet with cod-liver meal gave sub-normal growth

indicating the protein of this material to be of poorer biological value so far as growth is concerned. The amount of food required per unit of gain in body weight was much the greater in the cod-liver meal group and was comparable with that of gelatin. The mortality in the meat meal and fish meal groups was much greater than that with cod-liver meal and especially buttermilk powder. The losses from hemorrhage were confined to the meat meal and fish meal groups and particularly to the latter. A later paper will discuss this condition showing that with the same basal diet but with the fish meal unextracted by ether, no chicks were lost from hemorrhage and normal growth was obtained. Taking the mortality into consideration, the best results were obtained with buttermilk powder or casein.

There would appear to be little or no doubt that the leg-weakness which occurred with Groups I, VI and VII was in no way associated with the vitamin D content of the diet. Increasing the amount of medicinal cod-liver oil from 2 to 5 per cent had no effect nor was increased exposure to ultra-violet light beneficial. The bone ash and calcium and phosphorus content of the blood serum was quite normal. Varying the ratio of protein to yeast vitamin B within reasonable limits (Group XIII) had no marked effect on the condition, nor was the fibre content of the diet found to be associated with the incidence of this particular form of leg-weakness. The only significant difference between the diet with Group VI which developed extreme leg-weakness and the diet with Group XIV which grew normally was the substitution in the latter diet of white rice for paper pulp and dextrin.

Summary

1. Equal amounts of crude protein (N$\times$6.25) from buttermilk powder, fish meal or meat meal had practically the same effect on growth when supplementing a basal diet composed of marmite 15 per cent, cod-liver oil 3 per cent, bone ash to make the total ash of all the diets 4.36 per cent, and white rice to 100. Taking into account the high mortality with the fish meal and meat meal diets, the best results were obtained with buttermilk powder. Supplementing the same basal diet with an equal amount of crude protein from cod-liver meal gave subnormal growth, indicating the protein of this material to be of poorer biological value so far as growth is concerned. In all cases the protein supplements were freed from the variable amount of fat which they contained by extracting with ether.

2. An attempt to reduce the amount of extraneous nitrogenous substances in the basal diet by substituting ground paper pulp and dextrin for the white rice resulted in more or less complete failure in growth. Vary-

ing the amount of fibre (paper pulp) in the diet did not result in any significant improvement.

3. It has not been found possible to grow normal chicks on a diet in which the sole source of protein is of animal origin. The inclusion of white rice in the simplified basal diets used in these experiments had a pronounced effect in improving the growth, preventing the occurrence of leg-weakness, and reducing the mortality, which does not appear to be explainable from our present knowledge of the nutritional requirements of the growing chick. Some evidence is discussed which suggests that the effect is due to the rice protein and that some protein of vegetable origin, or some as yet unidentified substance, or substances, associated with vegetable material is necessary for normal chick nutrition.

Our thanks are due to Professor W. R. Graham and to Professor H. L. Fulmer for their interest and help throughout this work, the expenses of which were in part defrayed by a grant from the British Empire Marketing Board. We are also indebted to Mr. W. A. Munn, St. John's, Nfld., for supplying the medicinal cod-liver oil and the cod-liver meal used throughout these experiments.

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THE EFFECTS OF RADIANT ENERGY ON EXPERIMENTAL HEMOLYTIC ANEMIA*

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PREVIOUS observations (Laurens, 1928; Mayerson and Laurens, 1928), demonstrating that repeated irradiation of normal animals with the flaming carbon arc stimulates the hematopoietic system to a sustained production of a greater than usual number of red cells, encouraged the belief that such radiation might be particularly effective in stimulating blood regeneration in anemia. In a recent paper we (Laurens and Mayerson, 1931) report the results of irradiating 24 dogs made anemic by hemorrhage, and show that irradiation with the flaming carbon arc or the quartz mercury lamp produces marked and persistent increases in the number of erythrocytes and reticulocytes, particularly following long exposures, but fails to influence the regeneration of hemoglobin. Such experiments are a very severe test of the efficiency of radiation. Not only is hemoglobin removed from the body, but the diet used furnishes only a bare minimum of material which could be used in the manufacture of new hemoglobin and radiant energy cannot act as a substitute for dietary deficiencies. An hemolytic anemia affords a more favorable condition for radiation studies for there is but little loss of hemoglobin from the body and the retained hemoglobin may be utilized in the formation and maturation of new and functional red blood cells.

This report deals with the effects of radiation on dogs made anemic by the injection of acetylphenylhydrazine (pyrodin), which is just as effective in blood destruction as is phenylhydrazine hydrochloride but much less toxic to the liver (Bodansky, 1923; Long, 1926). The literature dealing with the experimental and clinical use of phenylhydrazine and its derivatives has been reviewed by Brown and Giffin (1926), Long (1926) and Giffin and Allen (1928). These compounds are strong reducing agents and split the hemoglobin into its pigment and protein fractions. The basic drug becomes oxidized, setting free a benzol ring which is the active agent in bringing about the blood destruction.

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The experimental procedures and methods have been given in earlier papers. The animals when received were bathed, purged and placed in large, roomy cages and fed the standard salmon bread diet of Whipple and Robscheit-Robbins to which additional salmon was added at times as an appetizer. Determinations were made of red and white blood cells, reticulocytes, hemoglobin, hematocrit, platelets, red cell fragility, specific gravity and total blood volume. When constant values had been reached, subcutaneous injections of various amounts of acetylphenylhydrazine were given. The original intention was to inject an amount of the drug sufficient to produce a severe anemia and to maintain the anemia at this level by further small injections. The development of a tolerance to the drug, however, made this method extremely difficult. Thus, the administration of 217 mg. (60 mg. per 100 cc. red cells) of pyrodin to Dog 8 produced in 7 days levels of 57 and 54 per cent of the normal for the red blood cells and hemoglobin respectively. A similar injection (212 mg., 60 mg. per 100 cc. red cells) one month later was followed by much smaller changes, the maximum low levels of 82 and 71 per cent normal in red cell count and hemoglobin, respectively, being reached in 19 days. A third injection, 6 weeks later, of 211 mg. (60 mg. per 100 cc. red cells) again produced only a similar slight grade of anemia (red cells = 79 per cent, Hb. 82 per cent normal) but in 22 days. Clinicians likewise report that a reduction dose may become a maintenance dose.

The dosage was calculated according to the total number of circulating red cells, the amounts injected varying from 40 to 100 mg. per 100 cc. of red blood cells (total amounts equal to from 104 to 876 mg.). In two experiments the dose was administered in two parts with a two day interval between the injections, in two others the dose was given in three successive injections. Single injections were employed in the remaining experiments. Determinations were made throughout the development of the anemia, and until pre-injection levels had been approached or reached. In several experiments the recovery proceeded to higher levels than before injection. With only one exception, the animals reacted favorably even when large amounts of the drug were injected. The one exception showed a very severe anemia (hemoglobin 15 per cent normal), and the animal was very weak and required careful attention for several days.

A total of twenty-four animals was used, five being exposed to the radiant energy emitted by a Cooper-Hewitt air cooled quartz mercury vapor lamp, eight to that emitted by a Pan-Ray flaming carbon arc burning "Sunshine" carbons, and the remainder serving as controls. The carbon arc, operated at 24 to 28 A and 50 to 60 V, emits an average of 0.820 gm.-

cal. per sq. cm. per minute of energy incident at 1 M. and has a distribution of 5 to 6 per cent in the ultra violet, 29 to 31 per cent in the luminous and 63 to 66 per cent in the infra red. The quartz mercury vapor lamp, operated at 4 to 5 A and 70 to 80 V, emits an average total energy of 0.0619 gm.-cal. per sq. cm. per min. incident at 1 M. with a distribution of 13 per cent in the ultra violet, 7 per cent in the luminous (400–1400 mμ) and 80 per cent in the infra red. In each case the abdomen and thorax were exposed. Various modifications of the period and time of exposure were used, the irradiation being started before the injection of the acetylphenylhydrazine, immediately after, or at the time at which the anemia was most pronounced. The exposures in the quartz mercury vapor arc series ranged from 15 minutes at 80 cm. (1.46 gm.-cal. per sq. cm.) to 30 minutes at 70 cm. (3.78 gm.-cal. per sq. cm); in the carbon arc series from 15 minutes at 80 cm. (19.2 gm.-cal. per sq. cm.) to 60 minutes at the same distance (76.8 gm.-cal. per sq. cm).

Twenty-four hours after the injection of acetylphenylhydrazine there was always some degree of anemia and this ran essentially the same course as illustrated by the figure. Under no conditions did the radiation have any apparent effect on the severity or rate of development of the anemia. The destruction of red cells after the injection of pyrodin was rapid for the first two or three days, and was most marked at the end of from five to nine days, the red blood cells and hemoglobin levels at this time being between 15 and 82 per cent of the original levels. The mean low levels in the twenty-four animals was 50 per cent, and the average period for the attainment of this level 8 days. The rate of the development of the anemia is slower when the dose is divided but single injections produce the same amount of destruction. The degree of anemia is in large measure regulated by the resistance of the cells of the particular animal or person to the drug and the regenerative activity of its hematopoietic system, rather than by the amount of drug injected. Thus the injection of equivalent amounts of pyrodin (277 and 297 mg. per 100 cc. red cells) into two animals of about the same weight (13.6 and 14.6 K.) produced a maximal anemia in 9 days in the first dog, the red blood cell count at this time being 49 per cent normal, while the second animal developed only a much milder anemia which reached its peak at the end of three days, the red blood cell count being 81 per cent normal at this time. The average number of red blood cells per cu. mm. of blood destroyed per day per mg. of the drug varies from 350 to 2000, the average of all the animals being 1200.

The sharp drop in the red blood cells during the first few days and the subsequent gradual decrease in their destruction suggests a strong and im-

mediate stimulation for the liberation of new cells. The end point of the anemia is reached when this production balances destruction. This stimulus to regeneration and liberation is indicated by the marked increase in the number of reticulocytes. The normal percentage varied from 0 to 3 per cent of the red blood count. Two or three days after the injection of pyrodin, this was increased in most cases to almost double, the count re-

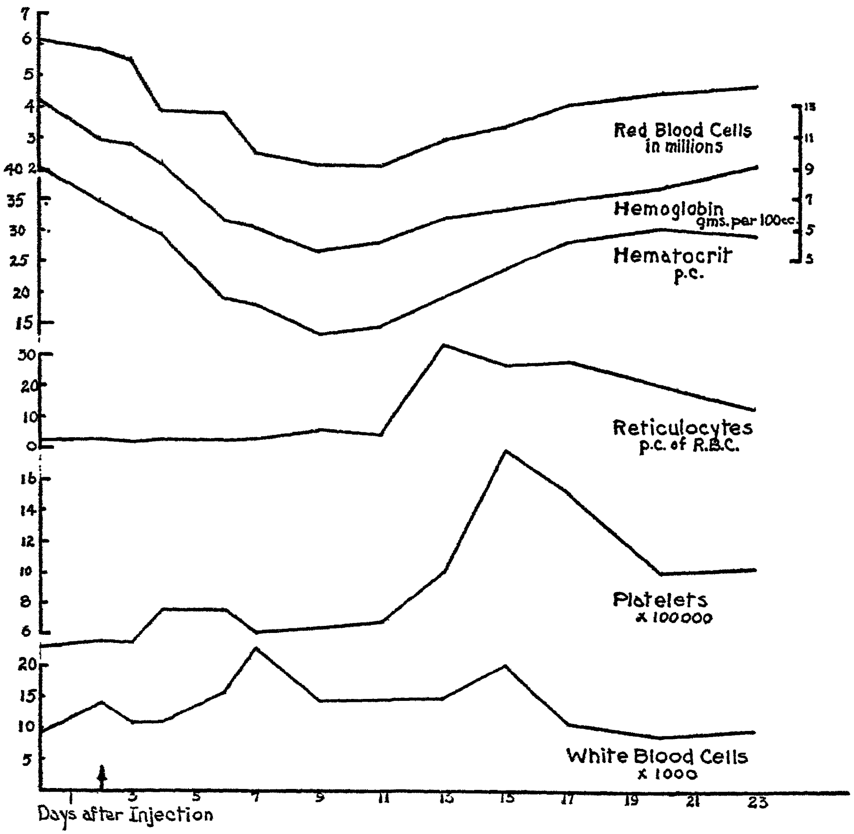

FIG. 1. Changes in blood cytology following injection of acetylphenylhydrazine (pyrodin). Dog. 4. Two subcutaneous injections of 233 mgs. each (40 mgs. per 100 cc. red blood cells); arrow indicates time of second injection.

maining at this level or increasing slightly. The first indication of recovery from the anemia was usually preceded by another sudden and marked rise. In ten experiments the reticulocytes were over 10 per cent of the red blood count at this time, the value reaching 33 per cent in one experiment. Further evidence of regenerative stimulation was shown by the marked spurts in platelets which occur along with the similar changes in the

reticulocytes. These platelet changes are transitory, the count soon returning to normal levels except in those cases in which the regeneration is particularly active. Thus in experiment 4, where the average reticulocyte and platelet counts before the injection were 3 per cent and 582,000 per cu. mm. respectively, there was a slight rise in both counts during the development of the anemia, followed by sharp supplementary increases to 33 per cent in the reticulocytes and 1,000,000 per cu. mm. in the platelets on the thirteenth day after the injection, at which time the red cell count was beginning to rise. The reticulocyte level gradually decreased as the number of reds increased, so that 10 days later the value was 13 per cent of the red count. The platelet level also showed a sharp drop but was still high at the end of the period of observation.

The curve of hemoglobin destruction usually parallels that of the red blood cells quite closely. Corpuscular hemoglobin, obtained by dividing the hemoglobin per 100 cc. of blood by the number of cells per cu. mm., is used as an index of the amount of hemoglobin in each cell. Corpuscular volume, calculated by dividing the volume of cells per 100 cc. by the number of cells per cu. mm. of blood, denotes the average volume of the individual cell. The corpuscular saturation, or actual proportion of the substance of each cell taken up by hemoglobin, is obtained by dividing the corpuscular hemoglobin by the corpuscular volume or directly by dividing the hemoglobin content expressed in gm. per 100 cc. by the hematocrit value expressed in cubic centimeters per 100 cc. Corpuscular volume usually showed marked increases and in twelve experiments the anemic level was 20 to 50 per cent greater than before the administration of the drug. This increase in the volume of the individual cells begins almost immediately after injection and persists throughout the recovery period. Values ranging from 70 to 95 cu. μ were frequently encountered, as compared with the average value of 59.3 cu. μ determined for 60 normal animals (Mayerson, 1930). During the development of the anemia, the corpuscular hemoglobin showed only minor fluctuations from pre-injection values, an indication that the hemoglobin liberated from the cells is not immediately available. This results in a lowered corpuscular saturation. In many of the experiments, the cells were only about 80 per cent saturated, while in some, values as low as 50 and 60 per cent saturation were obtained.

The destruction of the red cells and of the hemoglobin was accompanied by a rise in the icterus index, which, however, was not so great as would have been expected in view of the fact that the clinical use of phenylhydrazine often leads to jaundice. Determinations of serum bilirubin were negative in all cases, as Long (1926) reported in rabbits after pyrodin in-

jection, which indicates a low threshold and very rapid disappearance of the breakdown products of hemoglobin from the blood of the dog. During the first week after the injection of the drug, and particularly for the first few days after, the blood in many cases was decidedly darker in color than normally. During this period the hemoglobin calculated from oxygen capacity gave values lower than those obtained with the calibrated hemoglobinometer. Determinations by the Stadie method indicated the presence of some pigment other than hemoglobin, but spectroscopic examination for methemoglobin was negative, even when the blood was decidedly dark brown.

As the anemia developed, the resistance of the cells to hypotonic solutions of sodium oxalate increased, reaching a maximum either just before or at the point of lowest blood count and returning to normal during the recovery period. Various explanations have been proposed for this change, increased cholesterol content of the corpuscles, and increased amount of stroma among others. Perhaps the presence of large numbers of reticulocytes, which many believe to be quite resistant, is a contributing factor. Our experiments do not support the contention that the increased resistance of the red blood cells explains the acquisition of the tolerance to phenylhydrazine. In the three cases in which injections were repeated at intervals of 4 to 6 weeks, the fragility of the cells returned to normal before each subsequent injection, but the animals nevertheless showed a decided tolerance to the drug. It is possible that these cells may have been more resistant to pyrodin and not to sodium oxalate since Long (1926) was able to show changes in the resistance of rabbit cells to phenylhydrazine solutions but not to sodium chloride.

The changes in the leucocytes were irregular. In four experiments there was no change in the levels that could not be ascribed to normal variations. In nine cases there was a sharp rise (in one case to 33,000) following the injection which persisted for 1 or 2 days and was followed by a return to normal levels. In two of these experiments there was a secondary rise at the point where the red blood cell count was lowest. In the remaining nine experiments, the leucocyte count increased in inverse proportion to the red cell count, the highest counts (50 to 200 per cent normal) being found at the point of greatest blood destruction, followed by a return to normal. In two of these experiments the count remained high throughout the recovery period. Giffin and Allen (1928) also found an irregular response to the injection of phenylhydrazine HCl in dogs, and raised the question as to whether the leucocytosis was secondary, a result of tissue destruction, or primary, due to stimulation of bone marrow. Our experiments show both

types of responses. The first group, showing a sharp rise and decrease after the drug was injected, indicates a specific stimulus. Two of these animals, however, showed a secondary rise at the anemia level, which would suggest that this leucocytosis was due to secondary stimulation as a result of the destruction of red cell substance. The second group illustrates secondary stimulation, the increase in the number of whites being consequent to red cell destruction and proportional to it. The leucocytosis is generally due to increase in the number of the neutrophiles at the expense of the lymphocytes. There was no definite shift in the distribution in 6 cases, while 3 showed slight increase in lymphocytes and proportional decreases in neutrophiles. Normoblasts and transitional forms were common and in the severe anemias quite numerous, one animal (Dog 14), for example, having a transitional count of 1375 for two days. These forms disappear as soon as recovery begins, suggesting that the minimal level of the red cells was in part the result of bone marrow injury. Mononuclears and eosinophiles showed little change, and basophiles were seldom seen.

Although the radiant energy did not exert any definite influence on the development of the anemia, it markedly affected the rate and extent of recovery therefrom. The table illustrates the regeneration in sixteen animals, eight irradiated and eight controls, which developed corresponding degrees of anemia. The values are given in per cent of normal, a value representing the average of at least three separate sets of determinations made over a period of at least a week. For the sake of brevity and simplification, the table includes only the lowest value reached and the succeeding values at five day intervals.

The average anemia levels were the same for the two groups. There was somewhat more extensive hemoglobin than red cell destruction resulting in a corpuscular hemoglobin smaller than normal. Furthermore the volume of the individual cells increased and thus the corpuscular saturation was also considerably below normal in both groups. Five days later, the red cell number in both groups showed approximately the same amount of variation and increase. The hemoglobin regeneration was definitely better in the irradiated group, with an average gain of 17 per cent as compared with a 10 per cent gain in the controls. This difference became more marked as regeneration proceeded. At the end of ten days, the average red blood cell count in the controls had increased 20 per cent, while the irradiated group showed a gain of 30 per cent over the low anemic value. Similarly, the average hemoglobin value of the controls at this time was 25 per cent above the anemic level while the average value for the irradiated animals was 34 per cent above the low level, seven of the latter group showing values of

from 30 to 44 per cent higher. This difference in regeneration persisted. Fifteen days after the lowest level of the anemia the blood counts of the irradiated animals were from 80 to 96 per cent of the normal (except Dogs 9 and 12 which were only 72 per cent normal) as contrasted with those of the control group which were from 67 to 83 per cent normal, only two, however, showing values of 75 and 83 per cent respectively. The average hemoglobin level for the control group at this time was falsely high because of the unusual regeneration in one dog, No. 17, the values for the remaining animals being between 44 and 79 per cent normal. Two animals in the irradiated group (12, 22) showed similar low levels, the values in the others being 80 to 107 per cent normal.

COMPARISON OF REGENERATION OF CONTROL

Dog No.	Wt. Kg.		Red Blood Cells Per cent normal				Hemoglobin Per cent normal				Hematocrit Per cent normal			
			Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15
8	11.8	Controls	57	62	63	67	54	50	59	73	65	73	81	87
4	10.0	"	33	63	74		27	42	58	69	33	47	70	71
17	13.6	"	49	70	71	83	39	69	86	98	60	67	80	90
13	7.0	"	57	56	67	68	60	61	70	79	86	70	80	90
7	9.0	"	38	40	66	70	47	50	79		57	57	97	90
2	6.4	"	46	54	67	68	45	70	88	79	44	80	111	107
28	18.6	"	60	65	72	75	65	69	75	77	83	84	79	97
1	6.2	"	60	70	78	70	44	50	69	44	44	59	81	62
		Average	50	60	70	72	48	58	73	77	59	67	85	87
9	9.0	C Arc	54	80	98	72	68	81	88	90	77	108	114	108
12	9.0	"	35	50	64	72	25	53	59	70	47	47	61	80
19	10.6	"	44		88	86	41		85	93	52	92	97	97
24	8.6	"	52	61	76	92	44	57	74	80	63	69	93	91
22	12.0	Hg Arc	38	44	60	80	32	53	70	71	43	59	79	79
27	13.2	"	46	50	75	93	48	58	80	85	53	64	80	97
26	6.8	"	65	78	90	95	53	70	96	107	59	65	105	105
21	9.0	"	65	65	90	96	55	73	87	93	47	79		
		Average	50	61	80	87	46	63	80	86	55	73	90	94

The corpuscular volume remained greater than normal in both groups, but was more so in the control than in the irradiated animals. A probable explanation of this is that the newer cells appearing in the circulation of the latter as a result of the radiation were smaller and since regeneration was

more active in these animals the average corpuscular volume is lower than in the control group. The production of small cells following irradiation has been demonstrated in our previous studies. The fluctuations in corpuscular volume were similar in both groups, but the cells of the irradiated animals of smaller volume were more saturated than were those of the controls. The last two columns of the table show the average daily gains in red

AND IRRADIATED DOGS

Corpuscular Volume Per cent normal				Corpuscular Hb Per cent normal				Corpuscular Saturation Per cent normal				R.B.C. average daily gain for 15 days	Hb average daily gain for 15 days gms.
Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15		
113	120	129	128	89	89	88	110	83	80	72	85	73,333	0.18
101	100	109	96	81	91	81	80	80	92	94	83	167,333	0.35
126	105	120	114	83	99	123	119	65	102	100	110	158,667	0.42
140	107	116	120	97	96	98	103	66	85	84	84	78,000	0.16
152	145	146	128	123	126	119		80	108	80	100	134,667	0.26
91	149	171	160	107	123	124	108	131	95	85	79	82,667	0.20
136	130	107	128	106	105	83	101	79	81	78	79	73,333	0.11
75	80	104	87	74	74	89	90	98	85	85	103	46,667	0.15
117	117	125	120	95	100	101	102	84	91	85	90	101,833	0.23
143	134	116	130	126	100	90	93	88	74	80	73	130,667	0.11
137	96	105	96	73	107	100	105	50	111	95	109	145,333	0.32
121	135	110	108	82	85	86	100	69	64	86	93	160,000	0.46
121	122	122	95	83	102	96	83	66	84	79	88	146,667	0.24
110	133	131	121	86	121	109	109	62	90	84	90	153,333	0.34
123	137	109	111	110	121	113	98	89	88	104	86	200,000	0.40
90	83	117	110	83	91	102	112	90	109	87	103	100,000	0.41
73	81			72	105	91		111	109	147	115	100,000	0.22
115	115	116	110	89	104	98	100	78	91	95	95	142,000	0.31

blood cells and hemoglobin for the fifteen days following the lowest anemic levels. Not only were the averages in the irradiated group greater than were those of the controls but the individual values were distinctly higher. Only three control animals show an average daily gain of over 100,000 red blood cells, which is the lowest gain recorded in the irradiated group. Similar differences are apparent in the regeneration of hemoglobin. Specific gravity and plasma volume determinations show that these changes are real and not due to dilution or concentration of the blood. No significant

differences could be detected in the behavior of the reticulocytes, white cells or the resistance of red cells in the control and irradiated groups.

Our failure to obtain active regeneration of hemoglobin in our previous studies on hemorrhagic anemia and our success in these experiments on hemolytic anemia are due to the difference in the experimental conditions. A number of workers report that blood regeneration proceeds more vigorously in anemias caused by phenylhydrazine than in anemias due to hemorrhage in which the blood is actually lost. Injection of the lost blood has been claimed to remove this difference, the products of red cell destruction supposedly stimulating formation. Whipple and Robscheit-Robbins demonstrated that a very large portion of hemoglobin introduced either intravenously or intraperitoneally into the severely anemic dog may be conserved for the building of new and much needed hemoglobin. They further noticed that in most instances there occurs a certain lag in the output of hemoglobin following parenteral introduction of the blood pigment. One may believe that the hemoglobin is tucked away somewhere, or one may choose to assume that the hemoglobin is first broken down to end or intermediate products which are stored and used exactly as though these substances came from the digestive tract. Evidence seems to favor the latter view.

In a hemolytic anemia there is no actual loss of blood but instead a large store of liberated hemoglobin is made available. It hardly seems plausible that the hemoglobin would be utilized as such directly; but rather that it is broken down and worked over in some fashion or other for the rebuilding of new cell structures and hemoglobin. This process may take some time and this may perhaps be an additional cause for delay before regeneration can begin. In spite of this delay the final recovery is complete at an earlier date than that following hemorrhagic anemia. Itami and Ritz indicate that repair from phenylhydrazine injury is more rapid than that following loss of blood by actual removal. Price-Jones offers a very apt criticism of their experiments, since their conclusions were based on red cell and hemoglobin values and not on any estimate of total circulating hemoglobin (Robscheit-Robbins, 1929). Krafka (1930) also finds little or no difference in the rate of regeneration following hemolysis and hemorrhage, although Johnson and Berglund (1928) report an exceedingly rapid regeneration of the erythrocytes in phenylhydrazine anemia, with reticulocyte counts as high as 41.3 per cent.

The question as to the mode of action of radiation in speeding up regeneration is a difficult one to answer. The radiation certainly does not exert any marked detoxicating action on the drug after injection, for there is

little difference in the grade of anemia or in the time necessary for its development in the irradiated and control animals. This also rules out any greater secondary stimulation due to greater destruction. The cells in both groups show no significant differences in their resistance to hypotonic solutions of sodium oxalate. The effects of the radiation are on the recovery process after the most marked destruction is over. The experiments of Osato and Tanaka (1929) are interesting in this connection. They studied the effect of radiation on regeneration and iron metabolism in dogs, guinea pigs, and rats and state that the blood iron increased at the expense of the liver and bone marrow iron. The feeding of an iron-containing product,[1] resulted in deposits of iron in the spleen and liver which disappeared following irradiation along with marked blood regeneration. The authors conclude that following irradiation the reserve iron is withdrawn from the organs and is used in the manufacture of hemoglobin. The better regeneration following irradiation noted in our experiments may be due to a more efficient utilization of the iron made available by the destruction of hemoglobin. The action of radiation on the pigments set free may also be an important factor.

Summary

Anemia was produced in twenty-four adult dogs on a standard diet by the subcutaneous injection of acetylphenylhydrazine (pyrodin), the dosage being calculated in terms of red cell volume and varying from 40 to 100 mg. per 100 cc. of red cells. Eight animals were irradiated with the flaming carbon arc burning "Sunshine" carbons, five with the quartz mercury lamp and the remainder served as controls.

No significant differences in the rate of development or in the severity of the anemia were observed in the irradiated and non-irradiated animals. Some degree of anemia, coincident with an increase in the icteric index, was present in all cases at the end of 24 hours. The anemia became most marked in from 5 to 9 days, the red cell and hemoglobin determinations at this time showing values of from 15 to 82 per cent of the original levels. Single injections produced a similar degree but a more rapid development of the anemia than did divided doses of the same amount. Marked increases in reticulocytes and platelets occurred, and, in many cases, these constituents showed a further secondary rise at the beginning of regeneration. The corpuscular volume increased and remained high. The resistance of the cells to hypotonic sodium oxalate increased gradually and was greatest at the height of the anemia.

[1] Blutose.

The regeneration of the red cells and hemoglobin was unquestionably faster in the irradiated group, the carbon and mercury arcs being equally effective. The average cell and hemoglobin values of eight animals of the control group 23 days after the injection and 15 days after the lowest anemic level were 72 and 77 per cent normal respectively. The average values for the same number of irradiated animals that developed a comparable degree of anemia were 87 and 86 per cent normal. No significant differences in reticulocytes, whites, platelets and red cell fragility were observed.

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THE PHYSIOLOGICAL EFFECT OF RATIONS RESTRICTED PRINCIPALLY OR SOLELY TO THE ALFALFA PLANT

II. CYSTINE AS A LIMITING FACTOR IN THE NUTRITIVE VALUE OF ALFALFA PROTEINS*

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ALFALFA is grown so abundantly and cheaply in extensive regions of the West that the relatively high price of concentrates has led to the frequent practice of restricting dairy cattle largely to alfalfa. This practice and the fact that such cattle have at times (1) been found to be in negative nitrogen equilibrium, suggested the desirability of obtaining more definite information than we now possess on the biological value of alfalfa proteins. The extensive use of alfalfa as a consitituent of more or less well balanced rations has amply demonstrated its excellent feeding value but has not yielded specific information on the adequacy of rations in which alfalfa furnishes the principal or sole source of proteins.

The more limited number of experiments in which alfalfa furnishes the principal or sole source of nitrogen may be interpreted as indicating that alfalfa crude proteins possess a nutritive value that places them in about the same class as those of the corn kernel. Hart *et al* (2, 3) interested in determining the nutritive value of so-called non-protein nitrogen, concluded that the nitrogen of alfalfa hay possesses approximately the same efficiency for growth and milk production as that of the corn grain. Nevens (4) obtained a biological value of 62 for alfalfa proteins, when fed to rats at a 9 per cent level of intake. Just recently Sotola (5) reported a biological value of 56 for alfalfa hay proteins when fed to lambs at a level of protein intake equivalent to approximately 14 per cent of the dry matter consumed. Such data would seem to indicate that attention can profitably be given to a study of the factors limiting the nutritive value of the crude protein of alfalfa.

Several years ago (6) the writer obtained evidence indicating that the

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nutritive value of the crude protein of alfalfa hay is limited by its cystine content. In view of the difficulties incident to feeding rats such a bulky material as alfalfa hay, from which it has not been found practical to extract the proteins, we have fed products designated as "alfalfa leaves." The 1927, 1928 and 1930 products were obtained from second cutting alfalfa hay which had been cut when in about 1/4 bloom. The 1929 product was obtained from second growth alfalfa which had been cut when in the early bud stage. These products, hereafter referred to as "alfalfa leaves," contained varying amounts of buds, blossoms and stems.

Our general procedure has been to feed otherwise supposedly satisfactory rations containing enough finely ground alfalfa leaves to supply from approximately 9 to 15 per cent of alfalfa crude protein. The experiments described in this paper are of two types. First, a series of group experiments in which rats were most commonly fed in groups of 3, the food intake recorded for the group, and the individual body weights recorded weekly. Second, a series of paired-feeding experiments, as employed by Mitchell and Beadles (7). The group experiments will be described first.

Group Experiments

It was soon found that rations containing alfalfa leaves as a source of crude protein, fed *ad libitum*, produced very little growth. Of 21 rats fed *ad libitum* on rations 47, 47a, 57 or 57a (See Table I) for periods varying from 6 to 15 weeks, the maximum gain made by any one rat was 25 gm. over a period of 14 weeks. This rat was one of a group of 5, whose consumption of ration 47 averaged 44 gm. per rat per week. The average weekly gain per rat for the entire group of 21 rats was $0.32 \pm .13$ gm. The average weekly food intake per rat for the various groups varied from 31 gm. for a group of small (approximately 40 gm.) rats to 51 gm. for a group of approximately 70 gm. rats. The average weekly intakes for the various groups were 31, 44, 44, 44, 50 and 51 gm., respectively.

In order to assess the significance of the food intakes encountered, and to determine the suitability of the types of rations fed, various groups of rats were restricted to various levels of rations 51 and 54 or fed *ad libitum* on rations 51 and 59. The groups to which rations 51 and 59 were fed *ad libitum* may be dismissed with the statement that over periods of 6 to 8 weeks, with food intakes averaging 85 gm. per week, an average weekly gain of 19.8 gm. per rat was obtained. Three rats restricted to an average of 40 gm. per week of ration 51 made an average weekly gain of 8.6 gm. over a period of 9 weeks. Three rats restricted to an average of 40 gm. per week of ration 54 made an average weekly gain of 3.6 gm. over a period of 9 weeks.

TABLE I

COMPOSITION OF RATIONS IN PERCENTAGE BY WEIGHT

	Crude protein (N×6.25)	Ration number									
		44	47	47a	51	57	57a	59	64	67	70
1927 alfalfa	24.06	50.00	62.5	—	62.5	—	—	—	—	—	—
1928 alfalfa	19.38	—	—	62.5	—	44.0	—	—	—	—	—
1929 alfalfa	27.40	—	—	—	—	—	44.0	44.0	—	35.5	40.0
1929 alfalfa	30.00	—	—	—	—	—	—	—	40.	—	—
1930 alfalfa	23.06	—	—	—	—	—	—	—	—	—	**
NaCl	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$NaH_2PO_4 \cdot H_2O$	—	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.0
Na_2SO_4	—	—	—	—	—	—	—	—	—	—	.3
Starch	—	27.5	10.0	10.0	0.7	28.5	28.5	19.2	26.0	27.0	25.7
Sucrose	—	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	15.0	10.0
Salad oil	—	10.0	15.0	15.0	15.0	15.0	15.0	15.0	—	—	—
Butter fat	—	4.0	4.0	4.0	4.0	4.0	4.0	4.0	19.0	19.0	19.0
Cod liver oil	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dried yeast	47.34	—	—	—	—	—	—	—	1.5	—	1.0
Crude casein	78.48	—	—	—	9.0	—	—	9.0	—	—	—
1—cystine*	72.88	—	—	—	.3	—	—	.3	—	—	—
Total protein	—	12.03	15.04	12.11	22.32	8.53	12.06	19.34	12.71	9.73	11.43

* The cystine used in ration 46 was not tested for optical activity; most of that used in subsequent rations showed values for $[\alpha]_D^{20}$ varying from approximately −212° to −217°, as determined on 1% solutions in (N/I) HCL.

** 40% of 1930 leaves used from 25th to 35th days of experiment; from 35th day used 47.5% of 1930 leaves and 18.2% starch.

Ration No. 46 = 100 gm. No. 44+0.50 gm. cystine
Ration No. 48 = 100 gm. No. 47+0.05 gm. cystine
Ration No. 49 = 100 gm. No. 47+0.20 gm. cystine
Ration No. 54 = 100 gm. No. 47+0.50 gm. cystine
Ration No. 56 = 100 gm. No. 47+1.00 gm. cystine
Ration No. 60 = 100 gm. No. 57a+0.50 gm. cystine
Ration No. 65 = 100 gm. No. 64+0.20 gm. cystine
Ration No. 68 = 100 gm. No. 67+2.00 gm. 1929 leaves
Ration No. 69 = 100 gm. No. 67+0.30 gm. cystine
Ration No. 71 = 100 gm. No. 70+1.00 gm. 1929 leaves
Ration No. 72 = 100 gm. No. 70+0.25 gm. cystine

It has been our experience that the minimum energy requirements for the maintenance of 60 to 70 gm. rats are met by an intake of approximately 30 gm. per week of the type of rations under discussion.

Our first evidence indicating that the crude protein of alfalfa is deficient in cystine was obtained from a group of 5 rats that had failed to maintain themselves over a period of 8 weeks on an average weekly intake of 50 gm. of ration 44. During the next 10 weeks the ration of two of these rats was supplemented with 0.5 per cent cystine (ration 46), resulting in an average weekly gain of 9.4 gm. During the next 5 weeks cystine was omitted with the result that these rats merely maintained their weights on an average weekly intake of 84 gm. per rat. The 3 rats which were continued on ration 44 made an average weekly gain of approximately 1 gm. on an average weekly intake of 62 gm. per rat. After having been restricted to ration 44 for about 3 months, the latter 3 rats were observed to be eating hair from each other.

Of 31 rats variously restricted to or fed *ad libitum* on rations containing added cystine (rations 48, 49, 54, 56 and 60) not a single rat failed to make better growth than that made by the best rat receiving similar rations without added cystine. The rat which made the poorest growth on a ration containing added cystine gained 25 gm. in 9 weeks on a ration (ration 48) containing only 0.05 per cent of added cystine. A group of 3 rats fed *ad libitum* on ration 49 with an average weekly food intake of 63 gm. per rat made an average weekly gain of 7.2 gm. per rat. Still another group fed ration 54 *ad libitum* over a period of 13 weeks made an average weekly gain of 9.8 gm. per rat. A group of 4 rats fed *ad libitum* on ration 56 over a period of 8 weeks with an average food intake of 76 gm. made an average weekly gain of 12.5 gm. per rat. In another experiment two carefully matched groups, each containing 3 rats, were restricted for 8 weeks to the same average weekly intakes (47 gm.) of rations 57a and 60. The average weekly gain per rat on ration 57a was 1.8 gm. while that on ration 60 was 5.6 gm.

Paired-Feeding Experiments

At about the time that our group experiments were completed, Mitchell and Beadles (7) pointed out the advantages of Armsby's paired feeding method for amino acid studies. This method aims to restrict individual animals to the *ad libitum* food intakes of matched individuals. It is particularly well adapted to obtaining results,which can easily be subjected to statistical analysis, from a limited number of animals. Considerable difficulty was at first encountered in our attempt strictly to equalize the food

TABLE II
DATA OBTAINED BY PAIRED-FEEDING METHOD

Pair No.	Ration No.	Ration	Initial Wt.	Final Wt.	Total gain.	Total food	Weeks	"Comparison of weekly gains."
	64	Control	65	95	30	373	7	4
1.	65	Cystine	67	96	29	336	7	3
	64	Control	60	71	11	332	7	2
2.	65	Cystine	60	87	27	345	7	5
	64	Control	60	97	37	349	7	1.5
3.	65	Cystine	60	115	55	364	7	5.5
	64	Control	63	91	28	330	7	0
4.	65	Cystine	60	110	50	337	7	7
	64	Control	56	93	37	358	7	3
5.	65	Cystine	56	107	51	340	7	4
	68	Control	82	97	15	412	8	1
6.	69	Cystine	80	105	25	376	8	7
	68	Control	84	98	14	356	8	2
7.	69	Cystine	84	113	29	364	8	6
	68	Control	85	93	8	355	8	2
8.	69	Cystine	85	112	27	366	8	6
	68	Control	95	108	13	376	8	3
9.	69	Cystine	86	112	26	366	8	5
	71	Control	105	121	16	461	6	1
10.	72	Cystine	100	145	45	460	6	5
	71	Control	107	125	18	448	6	1
11.	72	Cystine	107	155	48	448	6	5
	71	Control	72	91	19	321	6	1
12.	72	Cystine	69	97	28	319	6	5
	71	Control	64	78	14	291	6	1
13.	72	Cystine	60	93	33	293	6	5
	71	Control	65	78	13	316	6	1
14.	72	Cystine	65	99	34	316	6	5
	71	Control	72	88	16	328	6	1
15.	72	Cystine	68	104	36	328	6	5

intakes of pair mates while at the same time allowing *ad libitum* consumption by the rat consuming the smaller amount of food. In the case of 3 pairs the food intakes of pair mates differed by 5 per cent or more, the difference, however, being in favor of the control rats. Since the rejection of experimental results is a dangerous procedure, all pairs are included in Table II.

The results obtained in our paired feeding experiments are not strictly comparable with those obtained from our group experiments. In our earlier work with rations free from added cystine, it was usually found that rats would lose weight during the first few weeks on the experimental rations. This initial loss in weight was largely overcome in our paired feeding experiments by a one week preliminary feeding period on rations similar to rations 51 and 59.

The rations used are described in Table I. The results obtained are summarized in Table II. All pair mates were matched for litter and sex. Pairs 1 and 2 came from one litter; 3, 4 and 5 from one litter; 6, 7, 8 and 9 from one litter; 10 and 11 from one litter; and 12, 13, 14 and 15 from one litter. It should be noted that pairs chosen from the same litter appear to show a decided tendency to make similar gains.

Of 15 pairs of rats fed rations with and without added cystine, the rat receiving added cystine made the greater total gain in all pairs except one. In this case (pair 1) the growth curves were practically identical in spite of the fact that the control rat was allowed to consume approximately 10 per cent more food than was consumed by the cystine rat. It therefore appears highly probable that cystine has effectively supplemented alfalfa crude protein as supplied by alfalfa leaves.

A summary of the "comparison of weekly gains" (7) is given in Table II. Of 103 such comparisons 78.5 favored the cystine rat, a deviation of 27 from the ideal mid-value of 51.5. The standard deviation of 103 such comparisons ($\sqrt{.25 \times 103}$) is 5.08. The deviation of 27 is therefore approximately 5.3 times the standard deviation, an outcome which cannot reasonably be attributed to chance.

From the data in Table II one may readily find the differences between the gains of pair mates, and from these differences, the average weekly differences in gain. The arithmetical mean of these differences is 2.56 gm. in favor of the cystine rats and the standard deviation ($\sqrt{\sum d^2/N}$) is 1.30. The ratio of the mean difference of 2.56 to the standard deviation, "Student's" Z (8, 9) is 1.97, an outcome which again clearly indicates that the nutritive value of our control rations was definitely improved by small additions of cystine.

Discussion

It should be emphasized that the results presented in this paper have been obtained over a period of about 3 years with a variety of rations and with 4 distinctly different lots of leaves. There can be no reasonable doubt concerning the effectiveness of cystine in improving the nutritive value of rations (for rats) in which the protein is supplied by alfalfa leaves. Whether our results with rats can be confirmed with farm animals remains to be determined. Our results are in harmony with the well known tendency for the proteins of legume seeds to be deficient in cystine. Moreover, our paired-feeding results are of the same order as those obtained by Mitchell and Beadles (7) for foodstuffs which are apparently well suited to the digestive tract of the rat. Furthermore, we have scattered data obtained by a slight modification of the methods suggested by Bergeim (10) and by Heller *et al* (11), which indicate a coefficient of digestibility of the crude protein of alfalfa leaves of approximately 60 per cent. The fact that considerable growth was obtained in some of our group experiments where we fed rations containing added cystine, likewise indicates that poor digestibility was at least not the principal cause of the poor growth obtained on rations without added cystine.

There is perhaps no more vexing problem than that of food intakes and their interpretation. The writer is of the opinion that there is no evidence in our records which indicates that cystine affected the palatability of the rations fed. This opinion is in harmony with the findings of Beadles, Braman and Mitchell (12) as well as with the fact that there is no good reason to suppose that a small amount of added cystine would appreciably affect the palatability of a ration containing some 40 to 50 per cent of alfalfa leaves.

Summary

Numerous group and paired-feeding experiments have been conducted with rats, using rations containing alfalfa leaves to supply the proteins. These experiments are interpreted as demonstrating a deficiency of cystine in the mixed crude proteins of alfalfa.

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DETERMINATION OF BASAL METABOLISM OF THE ALBINO RAT FROM THE INSENSIBLE LOSS OF WEIGHT

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NUMEROUS methods have been described for the determination of the basal metabolism of the albino rat. A large majority of these have been discussed by Benedict and MacLeod (1) when they described a modification of the closed circuit method used in the Carnegie Nutrition Laboratory. All of the methods are complicated and require elaborate apparatus.

Since it has been shown by Benedict and Root (2) that the basal metabolism of man may be predicted from the basal insensible loss of weight, and Levine, Wilson, and Kelly (3) found that the maximal variation of the insensible loss of weight of infants from day to day under rigidly standard conditions rarely exceeded 10 per cent of the average, and Johnston and Newburgh (4) found the insensible loss of a man, over a period of 37 days, to vary within 10 per cent of the mean, it appears likely that the insensible loss of weight may be used to determine the basal metabolic rate of small animals.

The insensible loss of weight, as defined by Isenschmid (5), is equal to the weight of the water vaporized, plus the carbon dioxide eliminated, minus the oxygen absorbed. Johnston and Newburgh (4) showed that the water of vaporization constituted from 82 to 104 per cent of the insensible loss of weight in man when the respiratory quotients were 1.00 and 0.707 respectively. Therefore, if the respiratory quotient is known, the weight of the water vaporized and the heat lost by vaporization of water may be accurately found by the determination of the insensible loss of weight.

In a study of the metabolism of normal adults in a respiratory chamber, Benedict (6) found that the heat eliminated by vaporization of water varied between 19 and 30 per cent and averaged 22 per cent of the total heat production; Soderstrom and DuBois (7), also using a respiratory chamber, obtained averages from 23 to 27 per cent in normal boys, adults, and elderly men. Levine and Wilson (8) using the same method, reported

from 21 to 29 per cent, with an average of 24 per cent, for six normal children, and later (9) found, for four normal and six marantic infants, an average of 27 and 26 per cent. Johnston and Newburgh (4) studied the total heat elimination by man over a period of 37 days, and found that 27 to 28 per cent of the total heat was eliminated by vaporization of water.

The insensible loss of weight has been used to calculate the oxygen consumption in the method described by Haldane (10) for the determination of the total respiratory exchange in small animals.

Recognizing the need for a simple, yet accurate, method for the determination of the basal metabolic rate of the albino rat, the heat eliminated by water of vaporization, calculated from the basal insensible loss of weight, was compared with the basal heat production, determined by the gaseous exchange.

METHOD

The insensible loss of weight was obtained by means of a balance sensitive to 0.5 mg. with a load of 300 gm. The animal was placed in a screen wire cage, constructed by inverting a six-inch kitchen sieve over a six-inch tin pie plate, which was suspended to one arm of the balance. In the bottom of the plate was a clean white filter paper covered with a coarse mesh wire. The balance was placed upon a table, and the cage was suspended beneath the table and attached to the balance by a strong cord which passed through holes in the top of the table and the base of the balance cabinet (as illustrated by Levine, Wilson, and Kelly (3) for determining the insensible loss in infants). The space beneath the table was enclosed, however, with a glass door on one side. This prevented any interference from air currents; allowed a light to be directed upon the rat in order to induce sleep; and prevented any disturbance of the animal by movements of the operator when the balance was manipulated.

The animals were placed in the screen cage without food or water from 18 to 24 hours before the experiment. This permitted them to become accustomed to the smaller quarters; they therefore went to sleep more quickly at the time of the experiment than when placed in the screen cage just prior to the test. Immediately before the experiment the animal was removed, the cage was cleaned (a new filter paper was placed in the bottom of the cage) and weighed. The animal was returned to the cage, which was then suspended upon the balance. After the animal had been quiet for 15 to 20 minutes, the first weight was obtained and the time noted. The period for the determination of the insensible loss of weight varied between the time required to lose 50 to 1500 mg. of body weight. In some instances the

insensible loss was determined before the gaseous metabolism, and, at other times, afterwards. The majority of the time, however, it was determined at the same time as the respiratory exchange, by determining the weight and noting the time just before the cage was gently lowered into the respiratory chamber. After obtaining the respiratory gases, the cage was gently elevated and suspended from the balance. As soon as the animal was quiet, the weight and time were again noted. The animal was then re-

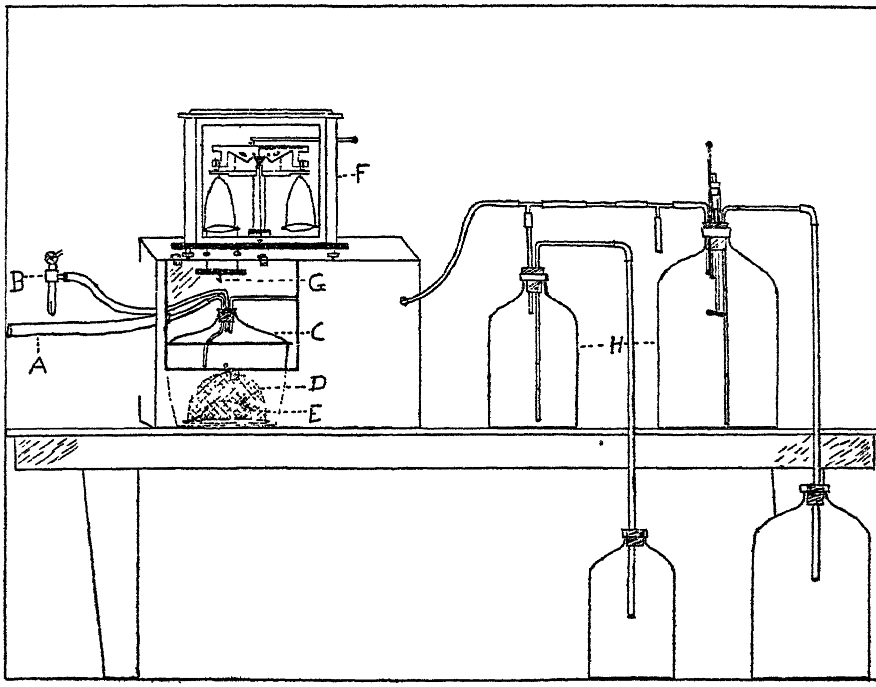

Fig. 1.

A. Hose for out of door air
B. Water suction pump
C. Vacuum desiccator
D. Screen wire cage
E. Animal
F. Balance
G. Hook for suspending cage to balance
H. Collecting bottles

moved and the cage weighed. It never changed in weight over 3 or 4 mg. unless the animal urinated or defecated, in which case the test was invalidated.

The gaseous metabolism was determined by a method similar to the one described by Prince (11), in which air was drawn through the animal chamber by the mechanism of a siphon and collected in a 20 liter bottle above the outflowing water, as shown in the diagram.

A large size vacuum desiccator was used for the respiratory chamber. The top was sealed with vaseline and could be easily removed, so that the cage containing the rat could be placed in the chamber or removed with only slight disturbance to the animal. Outside air, of a constant oxygen and carbon dioxide content, was first drawn through the animal chamber for thirty minutes at a rapid rate by means of a water suction pump. At the end of this time, the air in the chamber was repeatedly found to be of the same composition as the outside air.

The air was then drawn into a ten liter bottle for twenty minutes by the mechanism of a siphon (as described above). It was found by repeated analyses that this period of time was sufficient for the accumulation of carbon dioxide to a maximum for this rate of flow, which was usually about 1.0 per cent, never exceeding 1.5 per cent. It was then diverted to the twenty liter bottle at the same rate of flow as had been used for the smaller bottle.

The water used in the twenty liter bottle was triple distilled, and slightly acidulated by sulphuric acid. This was siphoned to another twenty liter bottle which was weighed immediately before and after the experiment,[1] and the temperature of the water recorded. The weight of the water was corrected to volume (12), and this gave the volume of air collected. The entrapped air was stirred for four minutes by a propeller attached to a mercury seal stirring rod. Then samples were analyzed with the Henderson gas analysis apparatus to an agreement of 0.04 per cent for the carbon dioxide, and 0.06 per cent for the oxygen. Alcohol checks gave respiratory quotients of 0.64, 0.65, and 0.653.

The animals had normal growth curves, and ate the diet[2] well. They were kept in separate cages which were cleaned and sterilized once a week. The temperature of the animal room was 27 degrees, Centigrade, constantly for three and one-half months.

Results

The influence of the respiratory quotient upon the percentage of the insensible loss of weight that is composed of water of vaporization is well illustrated by Rats No. 7 and No. 9. The data are shown in Table I.

Rat No. 9, on April 22, 1930, had a respiratory quotient of 0.707, and the water vaporized equalled 102.1 per cent of the insensible loss of weight;

[1] The weights were determined on a pair of silk scales (3) to an accuracy of 5 gm. for this weight.

[2] Diet consisted of corn starch 48 per cent, lard 25 per cent, dried pulverized beef 20 per cent, cod liver oil 3 per cent, and salt mixture 4 per cent. For vitamin B, four Harris yeast tablets were given daily. The salt mixture was that described by Osborne and Mendel.

TABLE I

HEAT PRODUCTION CALCULATED FROM INSENSIBLE LOSSES

Rat No.	Date	Wgt.	Age	Sex	Temp. °C	O_2 gm.	CO_2 gm.	R.Q.	Ins. Loss 24 hrs. (gm.)	Heat produc. 24 hrs. (Cal.)	Heat loss by vaporization of water	Per cent Heat loss by vaporization of water
5	4-18-30	184	4½	F	31	4.793	4.718	0.716	6.450	15.76	3.85	24.43
5	5-18-30	225	5½	F	28	5.209	5.125	0.715	7.810	17.12	4.59	26.80
6	4- 5-30	168	4	F	30	4.516	4.589	0.739	6.570	14.94	3.78	25.30
6	4-29-30	201	5	F	31	6.419	6.477	0.734	7.400	21.20	4.27	20.63
7	2-25-30	241	5	M	27	7.019	7.581	0.780	9.600	23.47	5.26	22.42
7	3-22-30	205	6	M	28	5.943	5.893	0.721	8.490	19.56	4.96	25.35
7	3-29-30	210	6	M	28	6.304	6.230	0.719	8.280	20.74	4.86	23.41
7	4-24-30	244	7	M	25	5.971	5.774	0.703	8.540	19.56	5.08	25.99
7	5-18-30	269	8	M	27	6.319	6.534	0.752	8.932	20.97	5.07	24.19
9	2-26-30	232	5	M	29	6.247	6.135	0.714	9.294	24.64	5.47	22.21
9	3-25-30	225	6	M	30	5.752	5.626	0.711	8.020	18.89	4.74	25.10
9	3-30-30	234	6	M	26	7.195	7.540	0.744	9.270	24.37	5.19	21.32
9	4-22-30	267	7	M	27	6.734	6.541	0.707	9.230	22.09	5.48	24.83
10	4- 1-30	205	6	M	28	5.477	5.421	0.719	6.720	18.03	3.94	21.88
10	5-14-30	285	8	M	29	7.040	6.784	0.801	8.640	23.05	5.18	22.45
10	5-21-30	297	8	M	30	6.875	6.670	0.705	8.926	22.55	5.31	23.56
11	4-25-30	475	8	M	30	12.456	12.043	0.702	16.000	40.85	9.55	23.93
12	5- 4-30	150	4	M	29	5.499	5.369	0.710	8.170	18.05	4.83	26.76
13	5- 2-30	161	4	F	29	6.065	6.200	0.743	7.319	20.90	4.18	20.81
13	5- 4-30	164	4	F	31	5.674	5.509	0.706	8.800	18.61	4.64	24.91

while, with Rat No. 7, on February 25, 1930, with a respiratory quotient of 0.78, the water vaporized equalled 94.1 per cent of the insensible loss. With each gram of water vaporized at 28 degrees, Centigrade, there are 0.582 calories given off.

Benedict and MacLeod (1) state that "with rats, the respiratory quotient is invariably not far from 0.72 or 0.73 when food has been withheld for sixteen hours." Mitchell and Carman (13) report an average of 0.739, while, in our series, the average respiratory quotient was 0.719. With the respiratory quotient averaging 0.719, there would be 100.8 per cent of the insensible loss as water of vaporization, as illustrated by Rat No. 7 on March 29, 1930. If the insensible loss were to be considered 100 per cent water, the calories lost by vaporization of water would be 4.82, whereas there were actually 4.86 calories, a difference of 0.04 calories. When the albino rat has fasted for from 18 to 24 hours, one may assume that the respiratory quotient is approximately 0.72 and that the insensible loss is composed of 100 per cent water of vaporization with the addition of an error of only 0.83 per cent.

The optimum temperature for the minimum heat production of the albino rat has been shown by Benedict and MacLeod (1) to be 28° C. Our environmental temperatures ranged between 25 and 31° C. without there being any constant change in the heat production or the percentage of heat lost by vaporization of water.

The total heat production of our animals compares favorably with that reported by Mitchell and Carman (13), and is somewhat lower than that reported by Benedict and MacLeod (1). The fact that our animals were asleep, and that they were unable to observe any movements of the operator during the experiment may account for our lower metabolic rates. Another thing that is thought to have lowered the heat productions is the fact that the animals had been in a constant environmental temperature of 27° C., for from two to three and one-half months. Animals No. 12 and No. 13, however, were brought in from the animal stock house, where the environmental temperature had ranged between 18 and 20° C. and their heat production compared favorably with that of the other animals.

The amount of heat lost by vaporization of water from the skin and lungs of the albino rat was remarkably constant as compared to the weight of the animal and the total heat produced. The percentage of heat lost by vaporization of water averaged 23.81 and varied between 20.63 and 26.80 per cent, with a maximal variation from the average of 13.3 per cent.

With Rat No. 5 on April 18, 1930; No. 6 on April 5, 1930; No. 10 on April 1, 1930 and No. 7 on March 22 and March 29, 1930, the total heat pro-

duction decreased markedly, due to the frequent fasting periods that accompanied the metabolic tests. They lost body weight and became quite thin, in accord with the experience of Benedict and MacLeod (1). It is interesting to note that the percentage of heat lost by vaporization of water by those thin animals was very close to the average.

An obese rat, No. 11, which Doctor C. A. Lilly very kindly allowed us to use, (one month older than our animals, but weighing 505 gm. before the twenty-four hour fasting period) showed a percentage of heat lost by vaporization of water very near the average. The lowered heat production in the female has been emphasized by Mitchell and Carman and by Benedict and MacLeod. This was confirmed in our series of experiments. The percentage of the total heat production that was lost by vaporization of water, however, did not show any constant variation between the two sexes.

Summary

1. The heat lost by vaporization of water and the heat production of the albino rat, in the basal state, have been determined under standard conditions of 18 to 24 hours without food or water and an environmental temperature of from 25 to 31° C.

2. The heat lost by vaporization of water averaged 23.81 per cent of the basal heat production of the albino rat, with a maximal variation of 13.3 per cent from the average, which held for normal, thin, and obese albino rats of both sexes.

3. Under these standard conditions, the respiratory quotient of the albino rat is about 0.72, and the water of vaporization is approximately 100 per cent of the insensible loss of weight. Insensible loss of weight per hour $\times 0.58 \times 100/25 \times 24$ equals calories per 24 hours.

We wish to express our gratitude to Doctor L. H. Newburgh for his interest in this work, and for his many helpful criticisms and suggestions.

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FACTORS WHICH DETERMINE RENAL WEIGHT*

**XII. THE NITROGEN INTAKE AS VARIED BY THE ADDITION OF UREA TO THE DIET

By

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IN THE preceding papers of this series (MacKay, MacKay and Addis, 1928; MacKay and MacKay, 1930) it was shown that when casein was the food protein the weight of the kidneys could be predicted from measurements of the casein consumption (casein intake = 0.0183 renal weight − 2.75). But the nitrogen of casein leaves the body almost entirely as urea and if this is the only mechanism underlying the casein-kidney weight relationship the same curve should be reproduced when urea is substituted for equivalent amounts of casein in the food. The experiments detailed in this paper were designed to test this hypothesis.

The first experiments of which we are aware, in which an attempt was made to increase the size of the kidneys by feeding urea in large amounts, were carried out in this laboratory by Addis and Shevky (described by Emge, 1921) on rats with[1] negative results in so far as any renal enlargement was concerned. Hinman (1923) also found no change in the size of the kidneys of rats following prolonged urea administration. At the time of their preliminary report dealing with the renal hypertrophy which ensues on high protein diets Osborne, Mendel, Park and Winternitz (1925) believed that comparable changes in renal weight could be produced by the inclusion of large quantities of urea in the diets. In their later experiments (1927) urea fed in large amounts, equivalent to the protein nitrogen concentration of the other diet, did not seem to lead to an equivalent enlargement of the kidneys.

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[1] This is somewhat surprising in view of the results reported here. The explanation seems to be in the fact that the rats were kept on the urea diet for a year and at the age they were killed the caloric requirement would have decreased and hence the urea intake would have been much smaller than at an early age (MacKay and MacKay, 1930). With the small number of rats which were used a minor increase in renal weight might have been easily overlooked.

TABLE I

Diet No.	No. Rats in group	Body length	Body weight (corrected)	Body surface	Heart Weight		Liver Weight		Kidney Weight	
					Actual	mgm. per 100 sq. cm. body surface	Actual	gm. per 100 sq. cm. body surface	Actual	mgm. per 100 sq. cm. body surface
		mm.	gm.	sq. cm.	mgm.	mgm.	gm.	gm.	mgm.	mgm.
	70 Days Old									
2	25	192	170	348	640	184	8.42	2.42	644	185
8	22	192	176	355	644	181	8.81	2.48	698	196
9	22	216	181	363	644	177	7.88	2.17	744	206
10	22	196	189	374	703	188	8.60	2.30	879	235
11	24	—	162	341	613	180	8.32	2.44	839	249
	400 Days Old									
2	22	231	367	581	949	163	12.89	2.21	1035	178
8	23	—	375	590	957	162	11.52	1.95	1031	175
10	10	—	333	543	953	175	11.63	2.14	1152	213
11	24	—	294	501	906	181	10.35	2.07	1030	209

TABLE II

Diet No.	Per Cent protein	Per Cent urea	Intake per 100 sq. cm. body surface per day: *Food	Protein	Urea	Urea nitrogen calculated as protein	Mgm. kidney per 100 sq. cm. body surface: Observed	**Calculated from protein intake	Deviation from observed	**Calculated from nitrogen intake as protein	Deviation from observed
			gm.	gm.	gm.	gm.			per cent		per cent
	70 Days Old										
2	18.0	0.0	3.39	0.61	0.00	0.00	185	183	− 1.1	183	− 1.1
8	18.0	4.0	2.90	0.52	0.12	0.34	196	179	− 8.7	197	+ 0.5
9	18.0	11.0	3.20	0.68	0.35	0.99	206	187	− 9.3	242	+17.5
10	31.3	11.0	3.20	1.00	0.35	0.99	235	205	−12.8	259	+23.0
11	18.0	18.0	4.74	0.85	0.85	2.42	249	197	−20.8	329	+32.1
	400 Days Old										
2	18.0	0.0	2.11	0.38	0.00	0.00	178	171	− 3.9	171	− 3.9
8	18.0	4.0	1.50	0.27	0.06	0.17	175	165	− 5.7	176	+ 0.6
10	31.3	11.0	2.11	0.66	0.23	0.65	213	186	−12.7	222	+ 4.2
11	18.0	18.0	1.82	0.33	0.33	0.95	209	168	−19.6	220	+31.0

* Determined from the average of the last 10 days of the experiment.

** Calculated by formula: *Protein Intake=0.0183 Renal Weight−2.75* (MacKay, MacKay and Addis, 1928).

The experimental methods have been described (MacKay and MacKay, 1927). The special diets which were used were prepared by replacing a portion of the cornstarch in diets which have been used before (MacKay, MacKay and Addis, 1928) with urea. This raised the nitrogen content of the diets accordingly and reduced their caloric value from 3 to 7 per cent. Growth during the period of observation was poorer on the urea containing diets in proportion to their urea content than on the control diet which

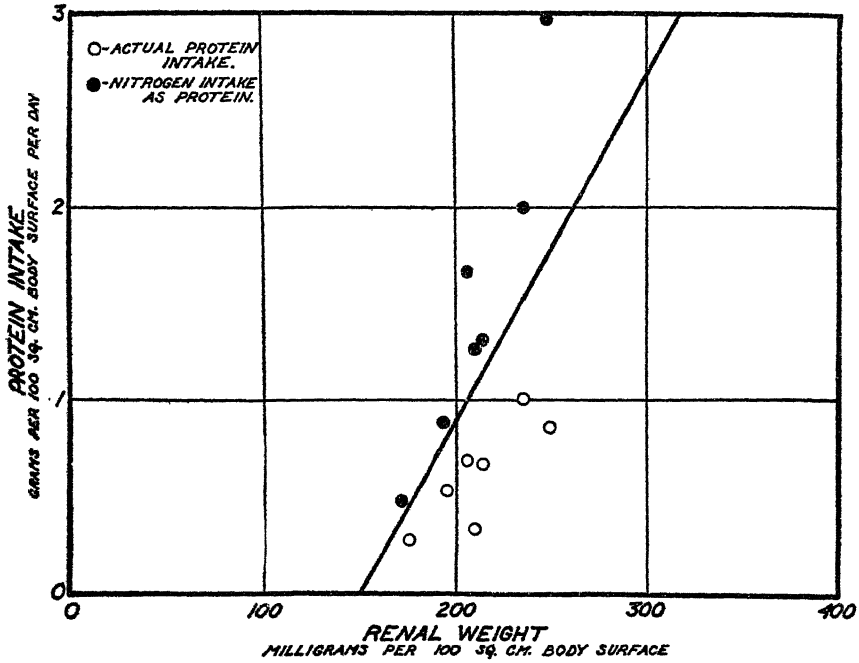

FIG. 1.

was free of this substance. Two groups of experiments were carried out, both on male rats of exact and known age. In one of these the rats were fed the special diets from 44 to 70 days of age and in the other from 346 to 400 days of age. These experiments were identical, except for the diet given, with those described for the protein diets. At 70 and 400 days of age respectively the rats were killed and anatomical measurements made. The average body weight and organ weights for each experimental group are presented in Table I.

It is obvious that the addition of urea to the diet is followed by a significant increase in renal weight. Our special interest is whether or not this is of the same order of magnitude as the increase in the size of the kidneys

which would be produced by the ingestion of similar quantities of nitrogen in the form of casein. To answer this question the data have been analyzed in Table II and Figure 1. In no experiment is the increase in renal weight as great in relation to the urea nitrogen ingested as the formula (MacKay, MacKay and Addis, 1928; MacKay and MacKay, 1930) demands for an equivalent amount of protein nitrogen. The difference is more significant than some of the actual figures indicate for we have assumed 100 per cent absorption from the intestinal tract of the nitrogen contained in both the urea and the protein. It seems certain that the urea nitrogen would be more completely utilized in so far as absorption is concerned than the protein nitrogen. We must conclude then that nitrogen in the form of urea added to the diet has less effect in increasing the weight of the kidneys than nitrogen in the form of casein.

Summary

The addition of urea to the diet leads to an increase in the weight of the kidneys of male albino rats 70 and 400 days of age. This increase in renal weight is less than that produced by the same nitrogen consumption obtained by the administration of protein instead of urea.

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GLYCOGEN FORMATION AND RESPIRATORY QUOTIENTS IN RATS FED EXCLUSIVELY ON FAT*

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IT IS generally agreed that the mammal is able to synthesize carbohydrate from protein, either in the normal or diabetic organism, and from glycerol in the diabetic state. Unanimity of opinion is lacking that either portion of the fat molecule may be converted into glucose in the normal organism. Pflüger (1907) was unable to recover any liver glycogen after feeding a dog 40 days on a pure fat diet. Bodey, Lewis and Huber (1927) were unable to demonstrate a deposition of glycogen in the liver of the rat fed 48 hours on butter fat. But Takao (1926), Burn and Ling (1928) and Magnüsson (1929) have pointed out that the rat on such a diet may deposit as high as 3 per cent liver glycogen. This deposition occurred only after 2 to 3 days of such feeding and increased thereafter. In all of these researches no analyses were made of the body glycogen content. These values may have decreased sufficiently to account for the increased liver deposits. Protein may also have been the source. But granted that the source is the fat administered, there is no way of telling, except by analogy with the diabetic, from which portion of the molecule the glycogen was derived.

If the fatty acid portion of the fat molecule were converted into carbohydrate at a sufficiently rapid rate, then at some time during its metabolism the R. Q. should exhibit a pronounced lowering, especially if the preformed glycogen had been reduced to a minimum by a preliminary starvation period and the carbohydrate were stored as glycogen. If glycerol were the cause of such sugar, the effect on the R. Q. would be negligible.

The object of the following experiments was to investigate the effect of fat feeding upon 1.–liver glycogen, 2.–total body glycogen, and 3.–the R. Q. during the absorption and metabolism of the ingested fat. From the data derived from these determinations it should be possible to make an accurate comparison between the administered fatty acids and glycerol and the glycogen deposition if such there should be.

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* Taken from a thesis submitted to the University of Rochester in partial fulfillment of the requirements for the Doctorate of Philosophy.

General Procedure

Thirty rats, divided into three control and three experimental groups, were used. Experimental and control groups were started together, so as to avoid differences due to season of the year. Young albino rats of 150 to 200 grams in weight and as far as possible of the same age and sex were placed on a constant diet for preliminary periods varying from a few days to six weeks. They were in the experimental room at least 24 hours previous to the start of the metabolism experiment. The rats were then starved approximately 24 hours. The control animals after starving one day were killed by a blow on the head and examined for their free sugar and glycogen content. The experimental group, after 24 hours fasting, was given butter fat. This was prepared by melting the butter, decanting it from its water three times and then filtering twice through a hot funnel to remove traces of casein. To the butter fat was added 3 per cent of Osborne and Mendel (1919) salt mixture. The rats were in special metabolism cages in which there was no scattering of food and no mixing or washing of the feces with urine. Daily records were kept of the weights of the animals, butter eaten and the urinary nitrogen excretion. The rats either ate the fat (group I) or were given it by stomach tube (groups II and III). They were kept in the metabolism trains 70 to 80 per cent of the time, being removed, as a rule, only for the passing of the stomach tube and urine collection. Two to three feedings per day were given and R. Q.'s were obtained immediately following and at varying periods after fat ingestion. One rat was starved 8 days and its carbohydrate content noted. Two others were given the saponifiable fraction contained in the butter fat. One rat was fed the potassium soaps obtained from butter fat. Experimental periods lasted from 3 to 9 days. The rats were then killed and analyzed in the same manner as the controls. Control sub-groups are numbered Ia, IIa and IIIa, experimental sub-groups Ib, IIb, and IIIb.

Analytical Procedure

Urinary Nitrogen. The urine was collected from the cages in 24-hour periods and analyzed by the macro-Kjeldahl method.

Carbohydrate Content. It was felt that a glycogen analysis on the whole animal was not sufficient to recover all the sugar in the carcass. Cori and Cori (1925), Palmer (1917) and others, have reported considerable amounts of free sugar in preparations analyzed with the utmost care to prevent glycogenolysis. Hence, different analytical procedures were tried in the three groups of rats in the effort to obtain the total sugars in the carcass, the same method in all cases being applied to controls and experimental sub-groups. In all three groups the initial procedure consisted in stunning the rat, immediately opening the abdomen and quickly removing the liver. The rat was then skinned and the carcass passed three times through a fine meat grinder. In group I the liver glycogen was determined by the method of Bierry and Gruzewka (1912), the hydrolysis being carried out in a boiling water bath and the proteins precipitated by the method of Folin-Wu (1928).

Applying this method to two liver brei mixtures, it was found to recover an average of 102 per cent of added C. P. glycogen. In groups II and III the liver glycogen was determined by Büttner's (1926) modification of Pflüger's method. In group III the glycogenolysis had previously been stopped by boiling the tissue in water and the free sugar extracted in 70 per cent alcohol. In group I the hide was analyzed by the method of Bierry and Gruzewka and the carcass free sugar by the method of Palmer (1917). In groups II and III the carcass and hide were analyzed together. Both were plunged into boiling water. After 15 minutes on a hot plate the beakers were placed on a steam bath and brought to a small volume. Alcohol, 95 per cent, was added to give a resultant concentration of 70 per cent by volume. The samples were placed in the ice box over night and next morning passed through a fine filter with repeated washings into volumetric flasks. Aliquots of the filtrates were evaporated to dryness on a steam bath, the residue taken up in 2.2 per cent HCl and hydrolyzed three hours in a boiling water bath. After neutralization the proteins were precipitated and the sugar determined. The residue on the filter paper and in the beaker were combined and analyzed according to the method of Pflüger (1910). The final sugars in all cases were rendered protein free by the method of Folin-Wu (1928) and determined by the Folin method (1929). In group I the true sugars were determined using Somogyi's technique (1928).

Respiratory Quotients. These were obtained on groups II and III by use of metabolism trains very similar to those described by Haldane (1892). Vacuum desiccators were used as the animal chambers. The animal was enclosed in a small wire cage contained in these and suspended over a tray for the collection of urine. Each set of absorbers was checked by another set immediately following it. These rarely showed any change in weight. In group IIb these experiments were carried out at a temperature of about 20°C; in group IIIb the experiments were done in a constant temperature room at 27 to 28°C. It is obvious that the activity of the rats in these chambers over long periods could not be controlled. This, however, has no influence on the R.Q.'s obtained. It only affects the total heat production. In all cases the activity was reduced to a minimum by allowing an electric light bulb to shine on the desiccators.

TABLE I

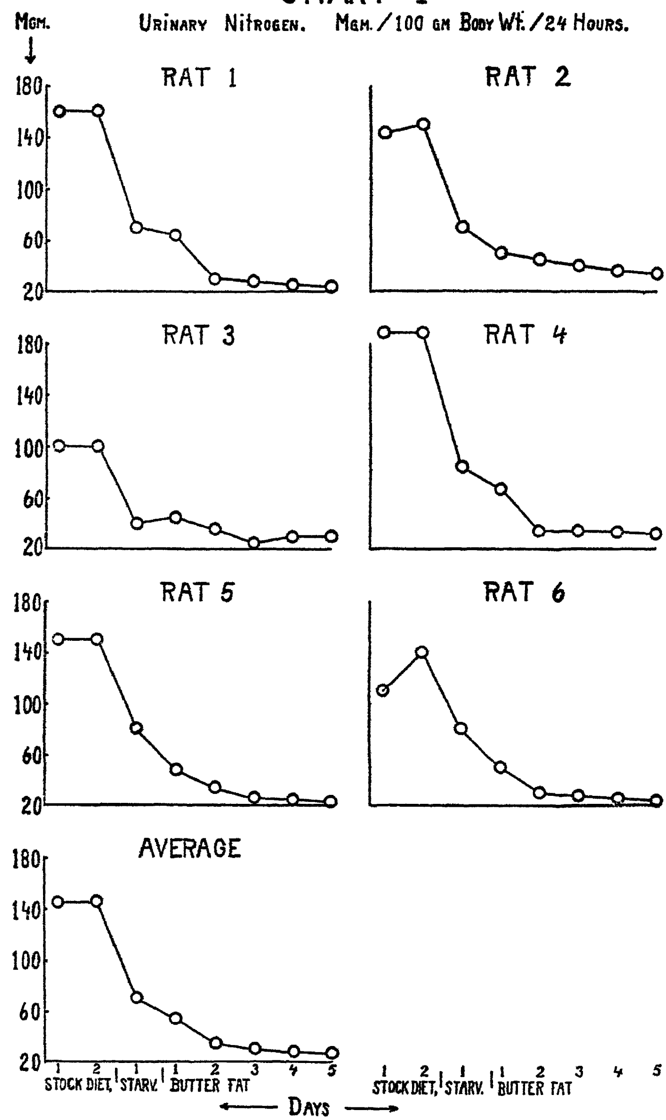
No. Rat	Initial weight	Days fat diet	% Decrease body weight	Fat intake gms.
		Group Ib		
1	170	4	2.9	19.6
2	171	4	2.9	17.9
3	164	4	4.2	13.5
4	180	4	0	26.6
5	200	4	0	29.6
6	200	4	5.5	18.9
		Group IIb		
1a*	198	8	25.7	0
4	204	6	11.7	21.6
5	173	7	10.4	26.1
6	174	8	5.7	28.7
7	222	3	5.8	16.8
8	188	8	8.5	32.6
		Group IIIb		
1	242	3	0	21.7
2	212	2	0	11.8
3	235	3	1.3	19.5

* Starved

Experimental Results

Body Weight. The animals lost weight in groups Ib and IIb. In group IIIb, in which the caloric intake exceeded the calculated caloric output, there was very little change in weight. Table I shows these weight changes together with the fat ingestion for all the rats which received fat.

CHART 1



Nitrogen Excretion. The urinary nitrogen was determined for all the experimental animals. Chart 1 shows the behavior of the total N excretion in the fat-fed rats of group Ib, this being typical of the other groups. Not all the curves obtained were as smooth but all showed the same marked diminution in excretion from day to day. The nitrogen excretion amongst members of the three groups, (Table II, column 10), is fairly constant. In group IIb, rat 1a was starved 8 days and its urinary nitrogen determined as well as its carbohydrate content. The nitrogen value, 45.8 mg. per 100 grams per 24 hours, is considerably higher than that for any of the fat-fed rats except rats 1 and 2, group IIIb. The sparing of body protein possibly illustrated here is due either to ingested fatty acids or glycerol, or both.

Tissue Analyses. These values for all three groups are presented in Table II. The average values per 100 grams of rat for the control sub-groups are 167, 147 and 126 mgm., with an average value for all three of 148 mgm $\pm$20 mg. Cori and Cori (1928) give an average of 143 mg. $\pm$11 mg. for 24-hour starved rats analyzed by Pflüger's method.

In sub-group Ib only 4 of the 6 fat-fed rats showed an appreciable rise in liver glycogen. It just so happens that these rats ate the largest amount of butter fat (Table I). When the total carbohydrate is expressed as mg. per 100 grams of rat, the average for the fat-fed rats on the basis of true sugar exceeds the controls by about 10 per cent. Expressed as total reducing substance, there is a loss of 4.8 per cent. If only the 4 rats which showed deposits of liver glycogen are averaged, there is an increase of 1 per cent or 22.5 per cent on the basis of the total reducing substance or true sugar respectively.

The rats in sub-group IIb are comparable with sub-group Ib. Rats 6, 7, and 8 show deposits of liver glycogen; rats 4 and 5 do not. All except rat 6 had diarrhea on the first day of feeding. Rats 4 and 5 appeared quite sick and died at an unknown time. This interval of time from death to analysis no doubt accounts for their low glycogen content. Taken together this group showed an increase of 25 per cent in total sugar content. If only those rats are averaged which deposited glycogen in their livers, the percentage increase is 41. These findings indicate in a more definite manner than in group Ib the absolute increase in body glycogen in such preparations. All three rats in sub-group IIIb exceeded the control sugar values, the increase being 39 per cent. Rat 1a, Group IIb, which was starved 8 days showed a glycogen value for the liver quite similar to the controls.

To obtain a rough idea as to the speed with which this glycogen was stored, one rat was starved 40 hours and given 2 grams of butter fat by stomach tube. Eight and one-half hours later, the liver glycogen value was

TABLE II

GLYCOGEN FORMATION AFTER FAT FEEDING
(ALL GLYCOGEN ANALYSES CALCULATED AS FREE SUGAR AND EXPRESSED AS MGM. PER 100 GM. RAT)

No.	Wt. rat gms.	Liver % body weight	Liver glycogen and free sugar	Carcass glycogen and free sugar	Total Reducing Sub.	No.	Wt. rat gms.	Liver % body weight	Urine N	Liver glycogen and free sugar	Carcass glycogen and free sugar	Total Reducing Sub.
Group Ia, Controls					Dec.	1929	Group Ib, Butter Fat					
1	200	2.6	10.7	138	149	1	165	2.7	35.8	43.9	157	201
2	188	2.9	11.3	180	191	2	166	2.7	41.3	11.1	120	131
3	195	3.2	13.9	172	186	3	157	4.1	33.7	15.2	134	149
4	176	2.4	15.7	161	177	4	183	2.7	37.2	42.2	123	165
5	204	2.8	12.1	122	134	5	201	3.7	33.4	59.0	107	166
						6	189	2.8	29.3	37.5	107	145
Av.	193	2.8	12.7	155	167±21		177	3.1	34.6±3	34.8	126	159±18
Av. (True sugar by yeast fermentation)			9.2	105	114±16					31.8	97	129±18
Group IIa Controls					Feb.	1930	Group IIb, Butter Fat					
1	169	3.5	4.6	150	155	1a*	147	3.0	45.8	10.9	150	161
2	154	2.7	2.3	124	126	4	180	5.2	39.3	4.8	126	131
3	218	3.0	10.8	106	117	5	155	5.3	44.2	12.0	152	164
4	158	3.0	4.7	147	152	6	164	3.6	34.8	67.2	169	236
5	146	3.1	6.4	171	177	7	210	4.3	41.3	32.8	156	189
6	186	3.2	11.3	143	154	8	173	3.9	43.5	48.5	149	198
Av.	171	3.1	6.7	140	147±17		172	4.2	40.6±2.8	33.1	151	184±29
Group IIIa, Controls					May	1930	Group IIIb, Butter Fat					
4	245	2.7	6.6	112	119	1	246	3.7	48.6	84.0	103	186
5	198	2.5	9.1	118	127	2	211	4.4		46.6	134	181
6	212	2.7	15.5	117	133	3	232	3.2	37.0	43.0	114	157
7	255	3.1	12.1	113	125							
Av.	228	2.8	10.8	115	126±4		230	3.7	43.7±5	57.9	117	175±12

* starved

3.12 mg. per gram of liver, a figure agreeing with the controls. Burn and Ling were also unable to obtain any appreciable glycogen deposition until the third or fourth day on such a diet.

No success was attained by feeding the saponifiable fraction of butter fat to two rats. The feeding was attended with violent diarrhea and was discontinued after 2 days. No analyses were made. The fraction fed was extracted as follows; butter fat was saponified with an excess of 25 per cent NaOH. This was acidified with HCl and the mixture extracted with petroleum ether. The solvent was later removed.

One rat was starved 96 hours and then allowed to eat the potassium soaps formed from the above preparation, the intake averaging about 4 grams a day. The liver glycogen was found to be 2.32 mg. per gram. From this single experiment it would seem that the glycogen deposition observed in the other two groups was due to the ingestion of the glycerol fraction rather than to the fatty acids. Further experiments are being carried out to clarify this point.

The R. Q.'s are set forth in Chart 2. All animals except rat 8 were kept in the metabolism chamber the major portion of the time. Of all the quotients obtained, only one—a 0.634 on rat 5—may be regarded as indicating sugar formation with fatty acids as its source. Ten figures lie between 0.66 and 0.70. These occur during the early hours of fat absorption and are due without doubt to a transient acidosis and ketonuria.

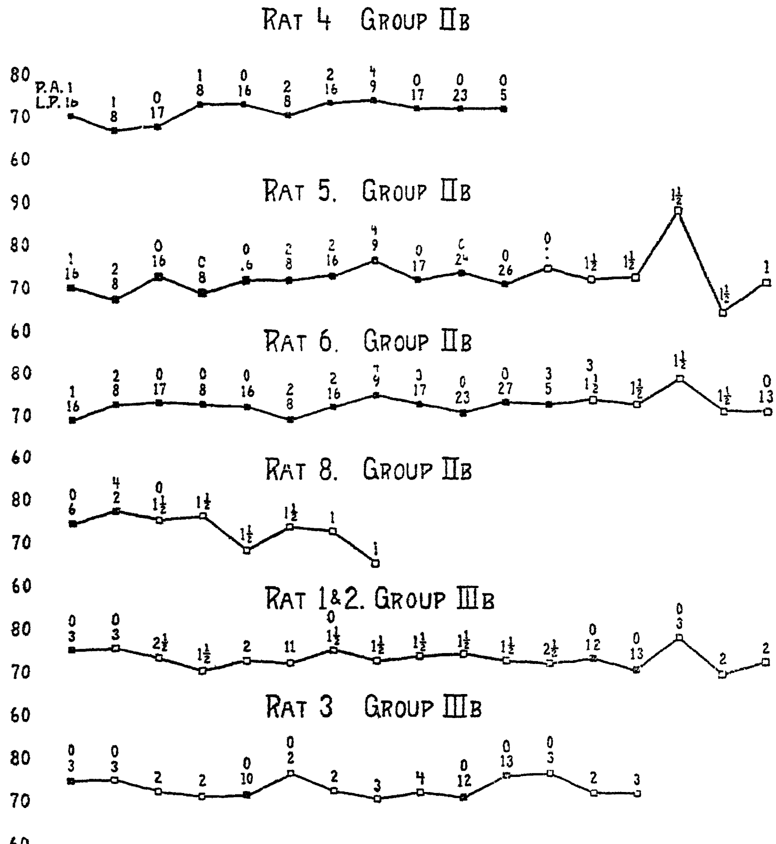
Rats 5 and 6 display a comparable series of quotients. On the seventh day of fat ingestion 2.5 cc. of butter fat were given by stomach tube and the R. Q.'s followed continuously in 1-1/2 hour periods from about the second hour after ingestion through the tenth hour. This was also done with rat 8. With the exception of one quotient each with rats 5 and 6, there is nothing to indicate conversion here. The remaining figures for rats 4, 5 and 6 average 0.726, 0.719 and 0.712 respectively. It should be pointed out that when this series of short, continuous quotients was made, the rats had already worked up a store of liver glycogen. Under such conditions any glucose formed from fatty acids might be oxidized in large measure as quickly as formed, in which case the R. Q. would be that of fat combustion.

This objection does not apply to group IIIb. Here continuous series of observations were obtained during the first fat feeding and thereafter. The basal quotients (post-absorptive) of 0.743 and 0.748 are high as compared with others obtained at a room temperature of 20°C., these averaging about 0.71 (not reported in this paper). The subsequent quotients during fat absorption average 0.729 and 0.732, respectively, figures both higher than in group IIb. There is no quotient below 0.700. In this series, if fatty acids

were being converted into sugar, then during the peak of absorption one might expect low R.Q.'s, indicating conversion and storage. Subsequently the quotients might increase to much above a fat level, indicating oxidation of the sugar formed. However, these quotients in general lend no support to the idea that the rat can store glycogen derived from ingested fatty acids.

CHART 2

RESPIRATORY QUOTIENTS AFTER FAT FEEDING.



ORDINATES NON-PROTEIN RESPIRATORY QUOTIENTS.
PA TIME AFTER ADMINISTERING FAT ORALLY TO NEAREST HOUR.
LP. LENGTH OF PERIODS IN HOURS.
□ CONTINUOUS SERIES OF QUOTIENTS DURING FAT ABSORPTION.

In Table III appears the heat production of the rats in groups IIb and IIIb, with its apportionment to carbohydrate, fat and protein. The total heat output per 100 grams of rat, per 24 hours, in group IIb of rats 4, 5 and 6, is fairly constant, the average being 19.12 ± 4.9 per cent. In group IIIb,

TABLE III
SUMMARY OF HEAT PRODUCTION

No. rat	Time of experiment	% time in metabolism chamber	Av. non-prot. R.Q.	Calories/100 gm. body wt.				Caloric intake/100 gms.	Total Cal. 100 gm./24 hours
				Carboh.	Prot.	Fat	Total		
		Group IIb—on Basis of Time spent in Metabolism Chamber							
4	140.85	79.0	0.707	3.29	6.13	111.19	120.60	119.0	20.54
5	174.0	78.0	0.718	4.06	6.99	125.07	137.23	164.6	18.92
6	186.0	73.0	0.720	6.51	7.12	125.05	138.83	179.8	17.91
		Group IIb—on Basis of Total Time of Experiment							
4	140.85	79.0	0.707	4.17	6.13	134.5	153.0	119.0	20.54
5	174.0	78.0	0.718	5.21	6.99	160.5	176.0	164.6	18.92
6	186.0	73.0	0.720	8.91	7.12	171.2	190.0	179.8	17.91
		Group IIIb—on Basis of Time Spent in Metabolism Chamber							
1+2	83.0	72.0	0.729	1.99	3.88	28.55	34.45	73.9	13.35
3	83.5	70.5	0.732	2.57	2.66	25.93	31.19	75.8	12.62
		Group IIIb—on Basis of Total Time of Experiment							
1+2	83.0	72.0	0.729	2.76	3.88	39.70	47.90	73.9	13.35
3	83.5	70.5	0.732	3.65	2.66	36.80	44.20	75.8	12.62

the average is lower, 13.00 ± 2.7 per cent. This is due, no doubt, to the higher environmental temperature. Only in group IIIb, did the calculated caloric intake exceed the calculated caloric output.

In Table IV an attempt is made to balance the deposit of glycogen and its oxidation against its various possible derivatives. Any R. Q. exceeding 0.707 was regarded as indicating sugar oxidation. Such an arbitrary mean is not wholly valid, for the quotient of the complete oxidation of butter fat is more nearly 0.720. On this basis there would be little or no sugar oxidized in these experiments as determined by the non-protein R. Q. What fraction of the total metabolism is due to the oxidation of ingested fat, as contrasted with body fat, cannot be told in these experiments. If the calculation be made in groups IIb and IIIb, however, the sum of the sugar oxidized as determined by the R. Q. plus the increase in body carbohydrate per 100 grams, averages 45 and 60.9 per cent respectively of that derivable 1.–from the ingested glycerol, 2.–plus that from protein metabolism and 3.–plus that from preformed glycogen. The average for both groups is 53.0 per cent. One and two-tenths, and 4.2 per cent, or an average of 2.7 per cent of the sugar derivable from the ingested glycerol, plus that due to protein, is recoverable as body glycogen in the groups IIb and IIIb. If, however, it is assumed that the rats in these two groups oxidized the same percentage of the three foodstuffs while out of the metabolism chamber as in it, and this period is taken into the calculation, then in groups IIb and IIIb, the values for oxidation plus deposit average 60 and 83 per cent respectively.

The marked depression of the urinary nitrogen excretion on the fat diet as compared with the previous 24-hour starvation period, is well brought out in these experiments (Chart 1). In the three groups the depressing action is 66 and 44 per cent in the first two groups, while it rose slightly in the third. It is very probable that a small fraction of sugar from protein has contributed to the observed formation of body glycogen. Such a process would tend to lower the R. Q. but in reality in these experiments the process is too small to have any effect. The ratio of actual sugars deposited to potential protein sugar is 11.5, 8.2, 0, 1.7, and 8.8 per cent respectively in rats 1 and 2 (which were in the same cage), 3, 4, 5, 6.

Discussion

From the preceding experiments a small but seemingly measureable deposit of glycogen in rats on a pure fat diet is the chief finding. The following table summarizes the carbohydrate analyses of all groups.

This deposit of glycogen was not attended with a depression of the R. Q.,

TABLE IV
GRAMS CARBOHYDRATE PER 100 GRAMS BODY WEIGHT

1 No. Rat	2 Carboh. oxidized from non-prot. R.Q.	3 Final body carboh.	4 Total carboh. (2+3)	5 From glycerol fed, gms.	6 Carboh. from prot.	7 Initial body carboh.	8 Total carboh. (5+6+7)	9 $\frac{2+(3-7)100}{5+6}$ %	10 $\frac{(3-7)100}{5+6}$ %
				Group IIb					
4	0.802(1.015)	0.131	0.933(1.146)	1.126	0.860	0.147	2.133	39.0(51.0)	0
5	0.890(1.140)	0.164	1.054(1.271)	1.557	0.980	0.147	2.684	35.7(45.5)	0.63
6	1.589(2.169)	0.236	1.825(2.300)	1.780	1.007	0.147	2.934	60.2(81.0)	3.00
				Group IIIb					
1+2	0.486(0.670)	0.183	0.670(0.853)	0.762	0.550	0.126	1.438	41.5(55.4)	4.6
3	0.627(0.890)	0.157	0.784(1.047)	0.444	0.376	0.126	0.946	80.0(111.0)	3.8

The figures in parentheses are based on the total time of the experiment.
The other figures are based on the percentage of time the rat was in the metabolism chamber.

such as might indicate conversion of fatty acids to sugar. This effect of fat feeding on body carbohydrate may be attributed to at least four causes; 1.–tissue dehydration, 2.–sugar formation from protein, 3.–sugar from glycerol and 4.–sugar from fatty acids.

It might be suggested that the loss of water from the tissues on a fat diet, combined with a transport of muscle glycogen to the liver, would explain the seemingly absolute increase in glycogen in the body of the rat. The criticism may apply to the tissue analyses but it has no bearing on the liver results which in the fat-fed average 23.8 per cent heavier than the controls and in addition contain two to three times as much glycogen.

TABLE V

	No. Rats	Mg./gm. liver	Mg./gm. rat	Remarks
Control	15	2.98	148.0	All rats included
Fat-fed	15	10.49	172.0	All rats included
Fat-fed	11	12.89	180.3	Rats showing no liver glycogen deposits excluded from average.

Muscle glycogen does not appear to have been transported to the liver in large amounts. In group IIb muscle glycogen determinations were made. The average mg. per cent in the two groups is very similar, the controls averaging 1.85 and the fat-fed group 1.92. Individual values for the two groups are 2.35, 2.35, 1.45, 1.45, 1.71 and 2.86, 1.12, 2.22, 1.47, 1.96 and 2.12 respectively. It is quite clear, then, that most of the observed increase in sugar is an actual formation of sugar.

That protein may be the source of this glycogen is quite possible. A portion of the carbohydrate from protein normally oxidized may be stored in this condition of carbohydrate deprivation. This would tend to lower the R. Q. and might aid in explaining the few low quotients obtained.

Sugar formation from glycerol is a reasonably safe explanation for these glycogen deposits. But the smallness of the storage and its slow rate of formation make it appear somewhat unreasonable to assume that its source is mainly glycerol. The latter substance, if fed to a carbohydrate-poor animal, is quickly and largely converted to glycogen. Since, however, the feeding of the soaps of butter fat had no effect on liver glycogen, one must conclude that the presence of glycerol is somehow associated with this process.

Can it be explained on the basis of a slow transformation of fatty acids

to carbohydrate? The very slowness of the process might be taken to indicate this. A process of such magnitude would affect the R. Q. within the probable error of the determination. Even the taking of quotients during the period of maximum absorption, which presumably coincides with maximum conversion and storage, would be of little aid in separating out such a process. For example, rat 1, in group IIIb, stored 148 mg. of glycogen. If it be assumed that during a 1-1/2 hour metabolism period one gram of fat is absorbed, then about 7 mg. of glycogen would be stored in excess of that deposited and oxidized. This rat, 246 grams in weight, in 1-1/2 hours would use about 470 cc. of oxygen at R. Q. of 0.71. To form the 7 mg. of glycogen would require an extra 2.5 cc. of oxygen and the R. Q. would be lowered to 0.707, a figure well within the experimental error of the method. Such a process, then, would have a negligible effect on the quotient.

The calculation is in keeping with the R. Q.'s obtained. These average 0.707, 0.718, 0.720, 0.729, 0.732 for the five rats in groups II and III, with an average of 0.720. There was little or no depression of the quotient during absorption. Certainly the quotients found in association with the small deposition of glycogen do not indicate that conversion is occurring.

No explanation can be advanced for the higher quotients in group IIIb as compared with group IIb. The glycogen content of the controls in this series averages even lower than in group Ib, which would seem to rule out as a possible explanation the oxidation of preformed glycogen during fat absorption.

Using, however, the R. Q.'s in group IIIb as a basis for calculation, it is found (Table IV, column 9) that the sugar formed and oxidized plus that stored is 83 per cent of that derivable from non-fatty acid sources. In group IIb the average is 60 per cent. These figures are without doubt much too high for they are founded on the assumption that R. Q.'s exceeding 0.707 indicate sugar oxidation. The true figure lies somewhere between 0.707 and 0.720.

Summary

1. Twenty-four-hour starved rats when fed upon a diet of filtered butter fat for three to eight days exhibit a gradual formation of liver glycogen averaging three to four times the concentration in control animals. The total glycogen content of the fat-fed rats exceeds the controls by 22 per cent.

2. The amount of glycogen deposited is equivalent to only about 2 per cent of the ingested glycerol and probably has its source in this substance.

3. One rat fed the soaps of butter fat failed to show any increase in liver glycogen over the controls.

4. In groups Ib and IIb there is a depression of the urinary nitrogen excretion as compared with the control 24-hour starvation period.

5. The average for all respiratory quotients is 0.720. The individual figures, during fat absorption, do not require an explanation other than direct fat oxidation.

6. There is no evidence in the foregoing data to substantiate the hypothesis that fatty acids can be converted into carbohydrate.

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FOOD INTAKE IN PREGNANCY, LACTATION, AND REPRODUCTIVE REST IN THE HUMAN MOTHER*

By

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WITH a better understanding of the basic principles of nutrition it has become evident (1 to 12) that every comprehensive study of the growth and development of the child should include some knowledge of the nutritional status of the mother preceding and during the reproductive cycle. Mendenhall (13) states that

> "During pregnancy, proper prenatal hygiene and feeding is essential to the life and normal development of the unborn child. To count on the fact that the mother is a factor of safety in the nutrition of the young, and to nourish the child either at the expense of the pregnant or nursing mother is an unnecessary sacrifice of the woman and may at any time prove disastrous to the child."

Furthermore, she continues,

> "there is every reason to believe that ability to produce breast milk of a superior quality is to some extent dependent on the storage of material from the mother's food during the prenatal period, as well as on the supply of nutrients from the food she receives during lactation."

Though it is acknowledged that the proper kind and amount of food is essential to the mother during pregnancy and lactation if she is to maintain good health and at the same time properly nourish her baby before and after birth, the literature is practically barren of any extended studies on the effect of diet on human reproduction, and the feeding of women during this crucial period still remains on an empiric basis. Our present knowledge

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of maternal nutrition therefore falls short of adequate information for the mother, and, indeed, beyond the point where practical experience avails, it is impossible to teach her how to live so as to produce a nutritionally stable child and at the same time conserve her own body tissues.

Sherman (14) emphasizes the fact that "too much weight must not be attached to any of the so-called dietary standards" which are at best "only an indication, not a rule." In this connection he quotes Hart, McCollum, Steenbock and Humphrey, who say

> "We need more effort placed on the accumulation of information on the physiological behavior of feeding stuffs than on the attempts to bring out new mathematical expressions of feeding standards."

Many current dietary standards for human reproduction, as a review of the literature indicates, have been stated empirically. The importance of the problem justifies a more thorough study of the nutritional needs of women during the reproductive cycle. The development of standards must include a more quantitative statement of the nutritional demands for growth and maintenance upon which the additional requirements for reproduction are superimposed.

It has been emphasized in previous publications (3, 4, 11, 15, 16) from these laboratories that there are many internal and external factors operating during pregnancy and lactation to influence maternal and fetal physiological processes, the significance of which bears directly upon nutrition but the intensity of which is little understood. For this reason it has been pointed out (3) that

> "It is the accumulation and recording of many and diverse data on the same individual and on different individuals in various stages of gestation that will ultimately reveal the functions and physiological processes peculiar to the progression of the human reproductive cycle."

As a part of a series of studies on the metabolism of women during the reproductive cycle and the factors operating to influence human milk secretion, dietary studies of the quality and quantity of food chosen by women in their own homes during and after a reproductive cycle have been made. The present report, which is one of a series on the dietary requirements of women during the complete reproductive cycle, contains a record of the calorie, protein, fat, carbohydrate, calcium, and phosphorus intakes of three women, whose physiologic processes are known to be well adapted to the requirements of pregnancy and lactation (3, 11, 15) at intervals throughout a complete reproductive cycle.

Procedure

The women subjects of this investigation were multiparae with superior capacities to produce milk. They were housewives living in their own homes and doing most of their household work. Previous publications (3, 4, 11, 15, 16) have recorded data on some of the physiological processes of these women during the reproductive cycle and serve as a background for the consideration of the dietary observations reported herein. All of the milk of these women was expressed by hand. The milk their babies did not take was sold to the Mother's Milk Bureau of Detroit. Subjects VI, VII, and VIII had an average daily milk output of 3134, 2366, and 1420 cc., respectively.

The dietaries were entirely self-chosen and included the foods the women liked and believed to favor milk flow. The dietary observation periods occurred at approximately monthly intervals throughout two lactation cycles of fourteen months' duration and the intervening pregnancies for Subjects VI and VII and throughout one pregnancy and the subsequent fourteen-month period of lactation for Subject VIII.[1] All foods were weighed and prepared in the home in such a manner that the amount of each ingredient consumed could be determined accurately. Approved methods (17, 18) of calculations were used to determine the essential food components that were consumed in the dietary.

Experimental Data

The maternal metabolism seems to be stimulated in response to the increased requirements for intrauterine growth and the concurrent formation of maternal tissue reserves in preparation for parturition and lactation. Demands over and above these are made in the process of the elaboration of milk, and, furthermore, immediately following the cessation of milk flow, that is, in the post-lactation period, the maternal body undergoes a stage of recuperation from the extended physiological demands of reproduction and an augmented food intake is maintained. In some species of animals there is a general increase in food consumption occurring simultaneously with the progression of the reproductive cycle, except immediately preceding parturition, when a slight depression is usually noted. The food intake remains high in the early part of reproductive rest for the purpose of rebuilding the maternal tissues that suffered losses during pregnancy, parturition, and lactation.

The above facts are demonstrated in the experiments of John and Schick

[1] Subject VIII showed a persistent albuminuria with no other apparent indications of ill health.

(19), Slonaker (20), Wang (21), and Goss and Schmidt (22), who showed that rats on a satisfactory diet increased their food intake somewhat during pregnancy and approximately trebled it during lactation. Additional data substantiating these findings are presented in Table I. These show

TABLE I

PERCENTAGE INCREASE IN FOOD INTAKE OF RATS DURING PREGNANCY, LACTATION, AND POST-LACTATION ABOVE THE PRE-GESTATION INTAKE

Rat No.	Pregnancy	Lactation	Post-Lactation
416	46	167	
1194	31	87	
867	20	116	−10
529	44	145	46
870	6	111	12
530	−10	78	
530	46	189	78
529	21	86	32
529	0	75	
659	32	139	30
929	42	185	
757	− 4	66	11
753	6	53	28
656	49	125	43
803	23	70	0
754	3	66	
1124	3	54	− 5
531	26	130	
531	8	106	
416	23	136	− 2
416	49	173	
417	10	113	33
756	− 6	77	6
755	20	164	10
889	− 4	63	
Average	19	111	21

that the food ingested by rats in pregnancy, lactation, and post-lactation, as based upon the intake over the three weeks immediately preceding conception, increased on an average of 19 per cent in pregnancy, 111 per cent in lactation, and 21 per cent in post-lactation. The requirements for pregnancy and post-lactation were approximately the same but the demands for lactation were appreciably greater than for pregnancy or the post-lactation period.

It is believed that the human mother's ability to produce breast milk of a

satisfactory quality is to some extent dependent on the storage of material from her food during pregnancy, as well as on the supply of nutrients from the food she consumes during lactation. The literature at our disposal reveals no extended observations on the food consumption of individual women during pregnancy, lactation, and reproductive rest. Information of the actual food intake of the individual woman in different stages of the reproductive cycle is therefore pertinent to a better understanding of the dietary requirements of women at this time and likewise to the establishment of dietary standards for maternity.

Observations upon three women whose food records are given herein are in accord with the findings respecting the food intake of rats during the reproductive cycle. In Tables II, III, and IV are recorded for Subjects VI, VII, and VIII, respectively, the average daily intakes of protein, fat, carbohydrate, calories, calcium, and phosphorus over three or more successive days at frequent intervals during pregnancy and lactation. To aid in direct comparison of one subject with another, these data are computed on a basis of unit body weight and summarized in Table V.

The women of this investigation had experienced long and continuous reproductive cycles. At the beginning of this study Subjects VI and VII were in their third and second lactation periods, respectively, and Subject VIII in her third pregnancy. Subjects VI and VII were followed through two lactation periods with an intervening pregnancy and Subject VIII throughout a complete reproductive cycle and in reproductive rest. Subjects VI, VII, and VIII bore infants with birth weights of 8 pounds 10 ounces, 9 pounds 3 ounces, and 6 pounds 12 ounces, respectively, and all infants grew at the rate of approximately 4 ounces per week during the initial forty-five weeks of life, the first twenty-six weeks of which they were given little food other than their mother's milk. All three women lactated for fourteen months with a milk production in excess of that needed for the nourishment of their babies.

It is common knowledge that some women gain in body weight during the lactation period, while others lose. Subjects VI and VII lost in body weight during lactation, while Subject VIII gained. A detailed discussion of the qualitative and quantitative aspects of food consumption in relation to nitrogen metabolism and physiological behavior in milk secretion will appear in later publications. The liquid intake has already been discussed in relation to milk flow and body weight (15).

In all three cases there was an augmented food consumption in lactation. From pregnancy to lactation the average daily calorie intake of Subjects VI, VII, and VIII increased from 3300 to 4200, 2900 to 4500, and 2600 to

TABLE II
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIET OF SUBJECT VI

Height 64.8 inches

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. Daily milk output (cc.)	Computed Average 24-hour Consumption						Nutritive ratio (1:)	Ca/P
					Cal.	Prot. (gm.)	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
					Third Lactation (A)							
1927 Dec.	60	10	63.1	1923	4600	172	193	534	2.92	3.43	5.6	0.85
					Fourth Pregnancy							
1928 Mar.	20	4	74.7	Birth Weight of Infant	3700	161	159	394	2.61	3.15	4.6	0.82
May	26	4	80.6		3000	126	137	326	1.93	2.40	5.0	0.80
June	30	4	84.2		3300	139	144	328	2.23	2.70	4.9	0.82
July	34	4	86.5		3100	129	134	357	1.76	2.33	5.0	0.75
Aug.	38	4	86.9		3200	110	122	406	1.90	2.21	6.1	0.86
Average for		20		8 lbs. 10 oz.	3300	133	139	343	2.09	2.56	5.1	0.81
					Fourth Lactation (B)							
1928 Sept.	7	4	73.1	3213	3800	149	149	462	2.60	2.99	5.3	0.87
Oct.	10	6	70.8	3167	4000	139	189	437	2.56	2.81	6.2	0.91
Nov.	14	5	68.8	3530	4600	172	221	476	3.31	3.66	5.7	0.90
Dec.	19	7	67.2	3450	3600	145	153	423	1.97	2.49	5.3	0.79
1929 Jan.	23	6	65.5	3547	4200	162	173	492	2.33	2.97	5.4	0.78
Feb.	27	4	64.7	3448	4600	177	191	543	3.28	3.61	5.5	0.91
Mar.	30	5	65.0	3534	4200	157	176	523	2.56	3.05	5.9	0.84
Apr.	34	6	65.0	3440	4400	159	202	507	2.83	3.25	6.0	0.87
May	38	4	64.7	3417	4800	174	219	544	3.09	3.48	4.5	0.89
June	42	4	64.0	3159	4000	137	195	431	2.41	2.72	6.3	0.89
July	46	1	64.7	2516	3200	160	122	370	2.70	3.19	4.1	0.85
Oct.	60	4	65.5	2856	4700	160	230	542	2.44	2.94	6.6	0.83
Average for		56		3134	4200	158	185	480	2.67	3.10	5.6	0.86
					Third and Fourth Lactation							
Average for		66			4200	169	186	484	2.69	3.12	5.7	0.86

Height 68.5 inches

TABLE III
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIETS OF SUBJECT VII

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. Daily milk output (cc.)	Computed Average 24-hour Consumption						Nutritive ratio (1:)	Ca/P
					Cal.	Prot. (gm.)	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
					Second Lactation (A)							
1927 Oct.	50	10	64.5	1197	4800	185	194	573	3.75	4.12	5.5	0.90
					Third Pregnancy							
1928 Feb.	14	4	66.3	Birth Weight of Infant	3300	114	133	422	1.66	2.22	6.3	0.74
May	26	5	72.1		3000	120	122	336	1.79	2.31	5.1	0.77
June	30	4	73.1		2800	125	122	307	1.70	2.44	4.7	0.70
July	34	4	75.0		2800	109	114	332	1.80	2.28	5.4	0.78
Aug.	38	4	75.6		2700	107	114	306	1.66	2.20	5.2	0.97
Average for		21		9 lbs. 3 oz.	2900	115	126	340	1.75	2.19	5.3	0.75
					Third Lactation (B)							
1928 Oct.	7	4	65.0	2683	4400	151	194	511	3.35	3.45	6.3	0.97
Dec.	13	4	68.0	2550	5600	216	182	754	3.16	3.84	5.4	0.82
1929 Jan.	18	3	66.5	2715	4600	148	135	713	2.99	3.71	6.9	0.81
Feb.	22	4	64.3	2444	3500	134	139	436	2.83	3.04	5.6	0.93
Mar.	25	4	63.4	2458	4600	167	197	527	3.16	3.70	5.8	0.85
Apr.	29	5	62.0	2485	4100	149	111	620	2.20	2.66	5.8	0.83
May	36	4	61.0	2506	5300	193	226	633	2.66	3.28	4.5	0.81
June	40	4	60.4	2284	4000	170	128	547	2.35	2.82	4.9	0.83
Nov.	62	3	61.8	1706	4600	139	168	683	1.73	2.48	7.7	0.70
Average for		35		2366	4500	163	164	603	2.71	3.22	5.9	0.84
					Second and Third Lactation							
Average for		45			4500	155	165	610	2.82	3.31	5.8	0.85
					Reproductive Rest							
1930 Feb.	11*	3	66.8		3500	97	139	463	1.10	1.64	8.0	0.67
Oct.	41*	9	65.8		4000	122	139	558	1.44	1.80	6.4	0.80

* Weeks after cessation of milk flow.

TABLE IV.
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIETS OF SUBJECT VIII

Height 62.5 inches

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. daily milk output (cc.)	Average Computed 24-hour Consumption						Nutritive Ratio (1:)	Ca/P
					Cal.	Prot.	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
					Third Pregnancy							
1928												
May	30	4	79.2	Birth Weight of Infant	2700	101	126	302	2.41	1.94	5.8	1.24
June	34	4	79.8		2400	78	98	308	1.53	1.46	6.8	1.05
Average for		8		6 lbs. 12 oz.	2600	90	112	305	1.97	1.70	6.3	1.15
					Third Lactation							
1928												
Aug.	7	4	70.4	1490	3800	165	195	356	3.71	3.62	4.8	1.12
Oct.	14	6	75.0	1468	3600	127	159	444	2.79	2.81	6.3	0.99
Nov.	18	8	76.7	1545	3700	141	128	443	2.53	2.80	5.2	0.90
Dec.	23	8	78.5	1550	4300	165	174	509	3.04	3.21	5.5	0.95
1929												
Jan.	27	4	80.8	1654	4000	160	211	410	3.43	3.40	5.5	1.38
Feb.	30	9	82.2	1449	4300	166	175	507	3.13	3.36	5.4	0.93
Mar.	34	7	82.1	1558	3800	149	170	417	2.93	3.01	5.4	0.97
Apr.	38	8	82.2	1515	3300	141	138	382	2.76	2.76	4.9	1.00
May	43	4	81.5	1518	4200	154	189	415	3.24	3.63	5.3	0.86
June	47	4	81.7	1418	3900	138	164	433	3.09	3.20	5.8	0.97
Sept.	63	4	81.4	1323	3900	155	179	420	3.40	3.31	5.3	1.03
Oct.	65	3	81.8	952	3300	147	150	347	2.95	3.06	4.6	0.96
Average for		59		1420	3800	151	170	425	3.08	3.18	5.3	1.01
					Reproductive Rest							
1930												
Jan.	13*	4	81.4		2500	85	92	326	1.47	1.54	6.3	0.95
Sept.	45*	9	80.8		2900	115	112	339	1.65	1.93	5.7	0.85

* Weeks after the cessation of milk flow.

TABLE V
AVERAGE DAILY FOOD INTAKE PER KILOGRAM OF BODY WEIGHT

Subject	VI			VII				VIII			
Reproductive Stage	Pregnancy	Lactation	Increase[1] in lactation, per cent	Resting	Pregnancy	Lactation	Increase[1] in lactation, per cent	Resting	Pregnancy	Lactation	Increase** in lactation, per cent
No. of Days Observed*	20	64		12	21	45		13	8	59	
Calories	41	65	60	59	41	70	70	34	33	49	49
Protein, gm.	1.7	2.4	45	1.8	1.6	2.6	59	1.3	1.1	1.8	59
Fat, gm.	1.7	2.9	71	2.1	1.7	2.6	52	1.3	1.4	2.1	49
Carbohydrates, gm.	4.4	7.4	69	8.1	4.8	9.4	99	4.2	3.8	5.2	36
Calcium, gm.	0.026	0.042	62	0.021	0.024	0.045	88	0.020	0.025	0.045	80
Phosphorus, gm.	0.030	0.047	57	0.027	0.031	0.052	68	0.024	0.022	0.043	95

* Each period of observation was three or more consecutive days in length.

** Per cent increase over pregnancy intake.

3800, respectively. Similarly, the protein intake increased from 133 to 158, 115 to 155, and 90 to 151 grams. There was also an increase in the average ingestion of other food essentials from pregnancy to lactation.

The per cent increase in the intake of various food essentials from pregnancy to lactation has been calculated on a kilogram body weight basis and recorded in Table VI. The average percentage for the three women

TABLE VI
PER CENT INCREASE IN FOOD ESSENTIALS IN LACTATION OVER THOSE CHOSEN IN PREGNANCY
(per kilo body weight)

Subject	VI	VII	VIII	Average
Calories................	60	70	49	60
Protein, gm............	45	59	59	54
Fat, gm...............	71	52	49	57
Carbohydrate, gm.......	69	99	36	68
Calcium, gm...........	62	88	80	77
Phosphorus, gm.........	57	68	95	73

TABLE VII
VARIATIONS IN QUANTITY OF FOOD CHOSEN BY TWO WOMEN DURING
TEN CONSECUTIVE DAYS OF MATURE LACTATION

Subject		Protein (gm.)	Fats (gm.)	Carbohydrate (gm.)	Calcium (gm.)	Phosphorus (gm.)	Calories
VI	Maximum	221	247	715	3.82	4.28	5500
	Minimum	147	134	416	2.18	2.81	3500
	Average	172	193	534	2.92	3.43	4600
VII	Maximum	240	279	719	5.30	5.33	5800
	Minimum	121	125	381	2.03	2.47	3100
	Average	185	194	573	3.75	4.12	4800

demonstrates the following increases: Calcium, 77 per cent; phosphorus, 73 per cent; carbohydrate, 68 per cent; energy, 60 per cent; fat, 57 per cent; and protein, 54 per cent. These data show that, as compared with the food intakes during pregnancy, lactation brought about an increased consumption of all dietary essentials.

There is a slight curtailment of food consumption near the close of pregnancy (Tables II, III, IV), as has been shown in the case of some animals (20). There was less activity as gravidity progressed, even though the women assumed the major responsibility of their housework and the care of

their families. However, there was no constant drop in food intake near the end of lactation. On the contrary, observations on Subjects VII and VIII during reproductive rest, three and eleven months after the cessation of milk flow, showed that the food consumption was practically the same as during pregnancy and that the augmentation of requirements in pregnancy and post-lactation were about the same. The gravid woman instinctively refrains from excessive bodily activity, and this inactivity, calling ordinarily for less than the average food intake, paralleled by the augmented food intake during pregnancy, doubtless obscures an increased demand for nourishment during the last part of pregnancy. Since these subjects had been continuously under the demands of the reproductive cycle for a considerable period of time, with long and intensive lactation periods, it is difficult to predict the recuperative demands in post-lactation and reproductive rest. Lactation in all three women increased the food demands for all dietary constituents approximately 60 per cent over and above those for pregnancy. Lactation, then, is truly the drain of pregnancy in a more intense degree (23).

The amount of food chosen and ingested by each woman varied greatly from day to day. The figures given in Tables II, III, and IV are averages for three or more successive days. The marked daily fluctuations in intake of the various food essentials in ten consecutive days of mature lactation, for Subjects VI and VII, are illustrated in Table VII. The maximum intake was at times more than twice the minimum. Thus the calorie intake for Subjects VI and VII varies from 3500 to 5500 and 3100 to 5800, respectively; similarly, protein ranged from 147 to 221 and 121 to 240 grams, respectively. The greatest variations occurred in the mineral constituents of the diet. Thus, the calcium intake varied from 2.18 to 3.82 and 2.03 to 5.30 grams and the phosphorus fluctuated from 2.81 to 4.28 and 2.47 to 5.33 grams, respectively, for Subjects VI and VII. As the maxima and minima indicate, the variations in the amounts of the different food components do not coincide, showing that the proportions of the different food components, as well as the amount of food, varied considerably from day to day.

From the data presented, the season of the year cannot be said to be a dominant factor in food consumption, since all three women had access to fresh fruits and vegetables throughout the year.

Summary

1. The food ingested by rats in pregnancy, lactation, and post-lactation, as based upon the intake over the three weeks immediately preceding con-

ception, increased on an average of 19 per cent in pregnancy, 111 per cent in lactation, and 21 per cent in post-lactation.

2. This paper records the intake of energy, protein, fat, carbohydrate, calcium, and phosphorus at intervals throughout a complete reproductive cycle for three women whose physiological processes are known to be adapted to the requirements of pregnancy and lactation.

3. The food requirements for pregnancy and for recuperation in post-lactation are of approximately the same magnitude.

4. There is a curtailment of food consumption near the close of pregnancy.

5. Lactation increases the food demands approximately 60 per cent over and above those of pregnancy.

6. There are marked daily fluctuations in food intake during lactation, the maximum being at times more than twice the minimum.

Note. An interesting report by Sandiford, Wheeler, and Boothby (24), giving the food intake of one woman from the 13th to 40th week of pregnancy, appeared after the completion of the present paper.

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GLYCOGEN AND FAT FORMATION IN RATS

V. CARBOHYDRATE-FREE DIETS

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THE experiments on carbohydrate-free diets were performed during the months of June and July, 1930. Fifty rats of the Long-Evans strain were taken at weaning time (3 weeks), and placed on McCollum's Stock Diet I for an observation period, during which time (1½ to 3 weeks) the gain in weight, daily food intake, and water consumption were measured. For the males, the mean initial weight was 46.8 grams, (range 36 to 69), the mean observation period 15.1 days, mean daily gain 4.1 grams, and the mean final weight 108.7 grams (range 83 to 145). For the females, the values found were 43.4 grams (range 32 to 59), 14.3 days, 3.0 grams, 86.9 grams (range 75 to 102), respectively. At the end of the observation period, 10 rats (6 males and 4 females) were placed on each of the 5 special diets and records kept as before. (Two rats developed infections, and were eliminated). At the end of 21 days on the special diets, the rats were killed, and liver glycogen (1) and liver fat determined.

The special diets contained lactose-free casein, lard and McCollum's "salt-mixture 185." Each diet was supplemented by daily vitamin feeding, as before described (1). The diets were composed as follows:

1.—87.5 per cent of the total caloric value in the form of lard, 12.5 per cent in the form of casein, the mixture having a caloric value of 7.07 per gram; 2.—lard 75.0, casein 25.0, caloric value of mixture 6.19; 3.—lard 50.0, casein 50.0, caloric value of mixture 4.95; 4.— lard 25, casein 75, caloric value of mixture 4.12; and 5.—lard 12.5, casein 87.5, caloric value of mixture 3.80 (Salt mixture 185–1 gram per 100 calories in each diet). The caloric value was reckoned as follows: by analysis, the lactose-free casein was found to contain 14.37 per cent of nitrogen. The theoretical value, according to Mathews, is 15.7 per cent. The casein as used, therefore contained 91.5 per cent casein, instead of 100 per cent. In making up Diet 5, for example, 2390.7 grams of casein, 138.9 grams of lard, and 100 grams of salt mixture 185, were used. $2390.7 \times .915 \times 4 = 8750$ cal., or 87.5 per cent of total. $138.9 \times 9 = 1250$ cal. or 12.5 per cent of total. The

total weight was 2629.6 grams, the caloric value 10,000; therefore each gram had a caloric value of 3.80.

Results

Preliminary Period

The mean daily caloric intake on the stock diet (3.88 calories per gram), daily water intake, daily gain in weight, and final weight at the end of the observation period, are shown in Table I. (The water intake was determined by subtracting the amount left in the bottle after a definite time from the original amount.)

Table I
Observation Period

Rats placed later on diet	Mean daily caloric intake	Mean daily water intake cc.	Mean daily gain in weight gms.	Mean final weight gms.
1	37.64	12.3	4.2	91.5
2	34.84	11.2	4.0	90.7
3	34.49	10.4	3.9	105.4
4	32.20	10.9	3.3	105.4
5	32.98	11.1	3.5	110.8

The results indicated that the rats were in good condition, as far as food and water consumption and gain in weight were concerned, during the observation period, and at the time they were placed on the special diets.

Results on Special Diets

When the rats were changed from the stock diet to the special diet, some adjustment was necessary; consequently, it seems best to present the results for each week separately. The results for water intake are shown in Table II.

Table II
Mean Daily Water Intake (cc.)

Diet	First week	Second week	Third week	Mean for entire period
1	7.5	8.1	8.3	8.0
2	11.6	13.3	13.5	12.8
3	11.4	13.5	15.6	13.5
4	8.7	13.3	18.0	13.3
5	10.9	15.3	18.5	14.9

It will be noticed that after adjustment (first week), the water intake increased in general, with increase in per cent of protein in the diet. On Diet 1, the water intake decreased 34.9 per cent below that on the stock diet, while it increased 14.3, 29.8, 22.0, and 34.2 per cent on Diets 2, 3, 4 and 5, respectively.

The mean daily caloric intake for each week is shown in Table III.

TABLE III

MEAN DAILY CALORIC INTAKE

Diet	First week	Second week	Third week	Mean for entire period
1	38.32	45.38	41.99	41.90
2	38.25	42.83	43.82	41.63
3	25.24	30.39	31.38	29.00
4	13.60	19.15	21.26	19.50
5	13.11	16.99	20.63	18.79

The caloric intake on Diet 1 increased 11.3 per cent above that on the stock diet; on Diet 2, the increase was 19.5 per cent. On Diets 3, 4, and 5, the caloric intake decreased 15.9, 39.4, and 43.0 per cent, respectively. Either the rats did not like the diets containing more than 25 per cent of the total caloric value in the form of casein, or less food was required.

Such a variation in caloric intake led to a similar variation in weight. During the first week, the most marked discrepancies in weight occurred, as would be expected. There were 5 types of response found. These were: 1.—straight gain in weight (9 animals on Diet 1, 9 on Diet 2, and 1 on Diet 3); 2.—some loss, then gain, until the weight at the end of the week was above that at the beginning (1 animal on Diet 1, 1 on Diet 2, 7 on Diet 3 and 2 on Diet 4); 3.—some loss, followed by some gain, the weight at the end of the week being still below that at the beginning (2 on Diet 3, 3 on Diet 4, and 6 on Diet 5); 4.—some loss, with the same amount of gain, the weight at the end being equal to that at the beginning (1 on Diet 4 and 1 on Diet 5); 5.—some loss, no gain, the lowest point in the weight curve occurring at the end of the first week (3 on Diet 4 and 2 on Diet 5). No losses occurred during the second and third weeks.

The mean gains in weight for all the rats on each diet are presented in Table IV.

In order to determine the relationship between the caloric intake and the gain in weight, the coefficients of correlation (ρ) were calculated. Table V presents the results.

With the exception of the third week on Diet 1, the relationship between the caloric intake and gain in weight is significant, regardless of the diet.

TABLE IV
MEAN GAIN IN WEIGHT

Diet	First week	Second week	Third week	Entire Period (21 days)
1	18.1	24.7	22.8	65.6
2	27.5	27.9	25.3	80.7
3	7.4	18.6	16.5	42.5
4	−8.3	12.7	12.1	16.5
5	−8.6	10.5	11.1	13.0

The absolute and the relative (expressed as per cent of body weight) liver weights, are shown in Table VI. The relative liver weights were first determined for the two sexes separately. Since no statistically significant difference was found between the two sexes on any one of the diets, the mean weights presented include both sexes. The differences between the

TABLE V
CORRELATION BETWEEN CALORIC INTAKE AND GAIN IN WEIGHT

Diet	First week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$	Second week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$	Third week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$
1	+.760±.090	8.4	+.744±0.095	7.8	+.329±0.19	1.7
2	+.702±.114	6.2	+.862±0.058	14.9	+.873±0.053	16.5
3	+.757±.091	8.3	+.532±0.15	3.5	+.788±0.081	9.7
4	+.757±.102	7.4	+.677±0.13	5.2	+.692±0.12	5.8
5	+.631±.143	4.4	+.792±0.089	8.9	+.627±0.14	4.5

TABLE VI
LIVER WEIGHTS

Diet	Mean absolute liver weights gms.	Mean relative liver weights per cent	Difference in relative weights—Diet 2 as subtrahend	$\frac{\text{Difference}}{\text{P.E. diff.}}$
1	7.48±.32	4.729±.126	+ .22±.15	1.5
2	7.77±.32	4.505±.087	—	—
3	6.98±.26	4.680±.097	+ .18±.13	1.3
4	6.82±.35	5.565±.170	+1.06±.19	5.6
5	7.14±.30	5.742±.074	+1.24±.11	11.3

relative liver weights on the various diets are also presented in Table VI. Diet 2 (25 per cent of the total caloric value in the form of protein) is used throughout as the subtrahend.

It will be noted that the liver is significantly heavier, in comparison to body weight, on the diets containing 75 and 87.5 per cent of the total caloric value in the form of protein.

The per cent of liver glycogen and per cent of liver lipid are shown in Table VII.

TABLE VII

Diet	Liver Glycogen (per cent)	Liver Lipid (per cent)
1	1.64±.10	12.80±.77
2	3.13±.17	5.62±.44
3	2.73±.10	4.13±.10
4	1.87±.14	3.05±.10
5	2.44±.10	2.94±.06

The optimum diet for glycogen formation was the one containing 75 per cent of the total caloric value in the form of lard and 25 in the form of casein (diet 2). However, even with 87.5 per cent of lard (diet 1), considerable glycogen was formed. The liver lipid content varied as the percentage of lard in the diet.

During the fall months of 1930, an entirely different type of experiment was used. Adult discard rats were used. The animals were fasted 48 hours, and then fed the test food for 24 hours and then killed. In order to establish standards, 9 female and 3 male rats were killed at the end of the 48 hour fast. The results are shown in Table VIII.

TABLE VIII
FASTING RATS (48 hrs.)

	Final weight (gms.)	Liver wt. (gms.)	Liver wt. as per cent of body wt.	Liver glycogen (per cent)	Liver lipid (per cent)
Females	194.3	4.620	2.397	.137	5.64
Males	303.0	7.519	2.477	.513	4.34

The most striking difference is in the per cent of liver glycogen. The difference is 0.376 per cent.

After determining the fasting glycogen level, rats were starved 48 hours,

then fed such test foods as stock diet, sucrose, lard, casein, and casein plus 20 per cent lard, for 24 hours, and then killed. The results are presented in Table IX.

TABLE IX
RATS STARVED FOR 48 HOURS, THEN FED 24 HOURS

Test Food		Final wt. (gms.)	Liver wt. (gms.)	Liver as per cent of body wt.	Liver Glycogen (per cent)	Liver lipid (per cent)
Stock	3 F.	194.2	7.444	3.87	5.20	3.11
Sucrose	3 F.	180.5	7.622	4.22	7.82	5.36
Lard	4 F.	178.2	5.295	2.96	.202	10.53
Lard	8 M.	260.4	6.446	2.56	.715	8.33
Casein	3 F.	173.3	5.628	3.26	1.95	3.53
Casein plus 20% lard	1 F.	176.0	6.974	3.96	1.88	4.64
Casein plus 20% lard	5 M.	262.4	9.061	3.48	1.81	3.51

Attempts were made to use the fatty acids from lard and their sodium soap as test foods. The rats ate such small amounts, if any, that the results are really those of a three-day fast. They ate glycerol, however, and formed glycogen, as will be shown. A few experiments were done on forced feeding. Oleic acid was given to one, and glycerol to several. The results are presented in Table X.

TABLE X

Test Food		Final wt. (gms.)	Liver wt. (gms.)	Liver as per cent of body wt.	Liver glycogen (per cent)	Liver lipid (per cent)
Sodium soap	3 M.	244.0	5.482	2.24	.190	9.40
Sodium soap	2 F.	179.5	4.037	2.26	.000	9.92
Fatty acids	2 M.	314.5	6.492	2.07	.260	11.14
Fatty acids	2 F.	206.0	4.515	2.22	.052	12.18
Oleic acid forced fed	1 M.	282.5	6.785	2.40	.207	4.61
Glycerol	7 M.	269.8	7.194	2.66	3.37	6.24
Glycerol	10 F.	213.1	5.898	2.78	2.63	7.37

I am unable to account for the sexual difference evident in the glycogen in the fasting rats, in those fed lard, sodium soap, fatty acid, or glycerol.

Summary and Conclusions

1. Young rats of the Long-Evans strain were fed test diets of lard, lactose-free casein and salt mixture 185 for 3 weeks, after a preliminary observation period. The composition of the diets was 1.—87.5 per cent of the total caloric value from lard and 12.5 per cent from casein; 2.—75.0 lard and 25.0 casein; 3.—50 lard and 50 casein; 4.—25 lard and 75 casein; 5.—12.5 lard and 87.5 casein.

2. The water intake was determined for each group on the stock diet, during the preliminary observation period and on the special diets. On diet 1, the water intake decreased 34.9 per cent; while it increased 14.3, 29.8, 22.0, and 34.2 per cent, on diets 2, 3, 4 and 5 respectively.

3. On diets 1 and 2, the caloric intake, as compared with the stock diet, increased 11.3 and 19.5 per cent respectively. On diets 3, 4 and 5 the caloric intake decreased 15.9, 39.4, and 43.0 per cent respectively.

4. The rats on diets 1, 2 and 3 gained steadily in weight, the mean total gain for the 21 days being 65.6, 80.7 and 42.5 gms. respectively. Those on diets 4 and 5 lost weight during the first week, but gained slowly during the second and third weeks. The mean total gain for the 21 days was 16.5 gms. for the rats on diet 4, and 13.0 gms. for those on diet 5.

5. There was a significant correlation between caloric intake and gain in weight, except in the rats on diet 1, during the third week of the test period.

6. The liver, in relation to the body weight, was significantly heavier in the rats on diets 4 and 5, than in those on the other diets. The livers of the rats on diets 1, 2 and 3 did not differ significantly from each other in relative weights.

7. The percentage of liver glycogen was 1.64, 3.13, 2.73, 1.87, 2.44 on diets 1,2, 3, 4 and 5, respectively. The glycogen formed on the first 2 diets may be partly from the casein and partly from the glycerol of the lard, as shown by later experiments.

8. The liver lipid content was 12.80, 5.62, 4.13, 3.05, and 2.94 per cent on diets 1, 2, 3, 4 and 5, respectively.

9. In later experiments, adult discard rats were fasted for 48 hours, then fed test food, during the last 24 hours before killing. The glycogen percentage dropped to 0.137 in female rats and 0.513 in male rats, on fasting 48 hours. Glycogen was readily formed by feeding the stock diet, sucrose, casein, or glycerol. Only a small amount was formed from lard. The experiments in which sodium soap and fatty acids were used as test foods were unsatisfactory, as the rats could not be induced to eat any amount. In

the one rat fed (forced) oleic acid, the glycogen content dropped below the fasting level.

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DISTRIBUTION OF MANGANESE IN FOODS*

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WITHIN the last few years it has been observed that a number of elements, although frequently occurring in only minute amounts, are universally present in plant and animal materials. Their universal occurrence can hardly be a matter of chance and, consequently, numerous investigators have attempted to ascertain what functions these elements perform in plants and animals. One of these elements, manganese, has received considerable attention from the standpoint of both plant and animal nutrition.

In 1913 Osborne and Mendel (1) observed that better growth of rats fed on "artificial protein-free milk" was secured when traces of iodine, fluorine, aluminum, and manganese were included in the ration. Daniels and Hutton (2), in 1925, reported that rats on a milk diet are able to produce five generations of normal young if small amounts of manganese, fluorine, and aluminum are added along with sodium silicate. The beneficial effects noted in these experiments, however, could hardly be attributed with certainty to the presence of manganese, since this element was only one of several added. Other investigations, especially in recent years, have been made in which manganese alone was added to various rations, some of which probably were inadequate from standpoints other than manganese deficiency. Levine and Sohm (3) reported that manganese produced a favorable effect upon rats as indicated by their appearance and activity as well as by a more rapid growth of their offspring. Richet, Gardner, and Goodbody (4) demonstrated that small amounts of manganese when administered to dogs every three days enhanced growth, whereas the reverse was true if the element was given more frequently. That manganese exerts a favorable effect upon the growth of mice and rats has been observed by Bertrand and Nakamura (5) and McHargue (6) respectively. McCarrison (7) likewise found that the element accelerated growth if given to the extent of 0.009 mg. per rat daily, but if the intake of manganese was increased

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to 0.56 mg. a definite retardation of growth followed. That the element is stored throughout life, but especially prior to maturity, and that the rate of storage can be markedly increased by the addition of manganese to either a complete or incomplete ration has been demonstrated by Skinner, Peterson and Steenbock (8).

With the discovery by Hart, Steenbock, and associates (9) that copper functions in hemoglobin formation, a new field of investigation was opened for the study of manganese as well as of various other elements. Titus and Hughes (10, 11) and Beard and Myers (12, 13) reported that manganese, like copper, is an active agent in the regeneration of hemoglobin, but the work of Hart, Steenbock, and associates (14, 15), Krauss (16), and Lewis and associates (17) throws the weight of experimental evidence against this conclusion.

Regardless of the final conclusion concerning the function of manganese in hemoglobin regeneration, most investigators are agreed that the element does play some important rôle in the animal organism. Therefore, in order that a sufficient amount of the element may be included in the human diet, a knowledge of its occurrence in various food materials is highly desirable. A previous publication from this laboratory (18) gave the manganese content of a number of our common food materials. More recently several investigators (19, 20, 21, 22) have contributed to the knowledge of the occurrence of this element in foods.

In this paper are presented the data for 83 food materials, the greater number of which have not been analyzed previously for manganese. The data given herein, together with those in a previous publication, have been combined so as to summarize concisely the occurrence of manganese in the common classes of foodstuffs.

Experimental

Since the method of preparation of the samples has been given in a previous publication (23), it need not be described here. The manganese determinations were run by the periodate method in which, with one exception, phosphoric acid was used for extraction of the ash. The procedure given by Davidson and Capen (24) was followed in the analysis of samples containing 0.1 mg. or more of manganese, which included mainly the vegetable materials. Foodstuffs containing very small amounts of the element were analyzed according to the modification used by the authors (25) in the determination of manganese in animal materials. The high chloride content of olives did not permit a determination of manganese by either of these procedures. Instead, the ash was extracted with dilute H_2SO_4, and

after the addition of 5 cc. of concentrated HNO_3 the chlorine was removed by evaporating until SO_3 fumes came off. To insure complete removal of the chlorine this step was repeated twice. The solution was then oxidized to permanganate and the color compared with the standard in the usual manner. In most instances, triplicate determinations, one of which involved the recovery of a known amount of added manganese, were made on each sample. If a recovery of 90 per cent or better was not obtained, the analysis was repeated.

Since it was the aim of this investigation to supplement the data previously reported, duplication of analyses was avoided. In two instances, however, it seemed desirable to check some of the results obtained in the previous investigation. In the former publication certain materials, *e.g.*, fish, were reported to contain either no manganese or only traces of the element. With a more sensitive method available, it seemed probable that the actual amounts of manganese in these materials could be detected and, accordingly, some of the samples have been reanalyzed. At the other extreme was a sample of blueberries which appeared unusually high in manganese. The original sample was reanalyzed and on more thorough oxidation was found to be even higher in manganese (209 mg. per kg., dry basis) than was previously reported (122.4 mg.). A new sample was therefore procured and was found to be still higher (314.9 mg. per kg.). From these results it appears that blueberries are normally high in manganese.

In Table I are given the moisture and manganese content, calculated on both the fresh and dry basis, of 83 representative food materials. It will be noted that in no case was the manganese concentration too low to warrant a numerical value, thus substantiating our belief that the more sensitive method would permit detection of the minute amounts present. On the fresh basis the concentration of manganese ranged from 0.028 mg. per liter of milk to 49.9 mg. per kilo. of bran flakes. Of the total number of foodstuffs analyzed, thirty-six were found to have a concentration of less than 1 mg., twenty-five from 1 to 5 mg., and only twenty-two more than 5 mg. of manganese per kilo of fresh material. On the dry basis the manganese content varied from 0.091 mg. in bacon to an exceptionally high concentration of 314.9 mg. per kilo. in blueberries. The six samples of milk varied from 0.017 to 0.048 mg. of manganese per liter. Three of these milks were produced by groups of the University herd on three different rations and three samples were obtained from herds in other states. Variations in manganese were less extensive among milks from the groups of the University herd than among milks from herds in other states.

TABLE I
MANGANESE CONTENT OF FOODS (EDIBLE PORTION)

Food*	Moisture	Manganese Content	
		Dry basis (100°)	Fresh material
	per cent	mg. per kg.	mg. per kg.
Almonds	3.9	20.2	19.4
Artichoke	84.4	23.2	3.6
Bacon	13.7	0.091	0.078
Beans, string (2)	91.4	28.2	2.4
Beef, casings	81.1	1.3	0.25
Beef, lung	80.3	2.7	0.52
Beef, pancreas	80.0	3.3	0.65
Beefsteak, T-bone	74.5	0.65	0.16
Blueberries	85.9	314.9	44.4
Brains, calf	80.7	2.3	0.44
Bran flakes	6.5	53.4	49.9
Brazil nuts	4.3	9.6	9.2
Bread, white	35.0	4.7	3.1
Butter	13.0	0.45	0.39
Cantaloupe	94.6	7.7	0.42
Cauliflower	93.0	25.2	1.7
Celery cabbage	95.0	23.3	1.2
Cheese, American	31.6	0.77	0.53
Cheese, cottage	75.0	2.1	0.53
Cheese, Swiss	37.5	3.1	1.6
Chestnuts, Italian	34.5	56.0	36.7
Chocolate, bitter	5.0	32.2	30.5
Cocoa	4.5	37.0	35.3
Coconut	46.4	25.0	13.1
Corn, sweet	68.0	4.7	1.5
Corn flakes	6.2	0.52	0.49
Corn-meal, white, prepared	6.2	1.6	1.5
Corn-meal, yellow, prepared	6.0	3.1	2.8
Cranberries	88.1	24.9	3.0
Cream of Wheat	7.4	4.8	4.5
Cucumbers	96.9	48.4	1.5
Dandelion greens	82.4	19.2	3.4
Eggplant	92.2	14.6	1.1
Eggs	72.8	1.1	0.30
Egg yolk	49.5	2.3	1.1
Endive	93.6	35.0	2.2
Filberts	2.0	42.8	41.7
Fish and sea foods			
Cod	80.7	0.63	0.12
Crab	77.1	1.3	0.30
Lobster	84.3	2.5	0.39
Mackerel	72.6	0.87	0.24
Oyster	90.0	20.8	2.1
Red snapper	79.2	0.63	0.13
Shrimp	71.0	1.0	0.29

TABLE I (*continued*)

Food*	Moisture	Manganese Content	
		Dry basis (100°)	Fresh material
	per cent	mg. per kg.	mg. per kg.
Flour, buckwheat	13.8	24.2	20.9
Flour, Graham	12.7	49.4	42.8
Flour, patent	12.0	4.5	4.0
Flour, rye	6.4	20.7	19.4
Hominy	9.7	1.2	1.1
Honey	12.6	0.36	0.31
Lamb chops	69.2	1.2	0.37
Lemons	88.9	3.6	0.40
Lettuce, leaf	93.8	167.0	10.4
Milk, whole			
Maximum		0.37	0.048**
Minimum		0.13	0.017**
Average (6)	87.5	0.22	0.028**
Milk, colostrum	85.1	0.28	0.044**
Milk, whole, powder	5.0	0.19	0.18
Molasses	25.7	5.5	4.2
Mushrooms	88.1	6.9	0.82
Oatmeal	9.9	31.0	27.9
Olives, green, canned	77.0	2.4	0.55
Oyster plant	72.0	12.4	3.5
Peas, split	9.5	30.7	27.7
Pecans	2.3	35.7	34.8
Peppers, green	92.5	19.1	1.4
Plums	88.0	6.2	0.74
Pork chops	63.1	1.6	0.59
Poultry			
Duck	43.7	0.58	0.33
Goose	57.0	1.2	0.52
Turkey, dark meat	72.2	1.6	0.45
Turkey, light meat	72.1	1.1	0.31
Puffed Rice	10.9	8.4	7.3
Puffed Wheat	8.6	30.2	27.2
Pumpkin	91.7	4.5	0.37
Radishes	96.2	13.7	0.52
Raspberries	88.9	45.9	5.1
Rhubarb	95.3	30.6	1.5
Rice, polished	13.3	12.9	10.8
Rutabagas	86.6	9.9	1.3
Shredded Wheat	8.1	25.9	23.9
Tomatoes	94.2	24.0	1.4
Turnips	92.0	5.4	0.43
Veal chops	78.5	1.3	0.28
Watercress (2)	92.2	69.1	5.4

* When more than one sample was analyzed, the number of samples is indicated by the figure in parentheses.

** mg. per liter.

A survey of the concentration of the element in classes of food materials is obtained from Table II. In this table are listed 12 major classes of foodstuffs, arranged in descending order of their manganese content, together with the minimum, maximum, and average for each group. This table, as mentioned above, has been compiled from the data in Table I and those in the previous publication (18) wherever numerical values were obtained for the manganese concentrations.

TABLE II

GROUPS OF PRINCIPAL FOODSTUFFS ARRANGED IN DESCENDING ORDER OF MANGANESE CONTENT—FRESH BASIS (EDIBLE PORTION)

Class of food	Number of samples	Manganese		
		Minimum	Maximum	Average
		mg. per kg.	mg. per kg.	mg. per kg.
Nuts	10	6.3	41.7	22.7
Cereals and their products	23	0.49	91.1	20.2
Dried legume seeds	4	10.7	27.7	20.0
Green leafy vegetables	18	0.76	12.6	4.5
Dried fruits	7	1.5	6.7	3.3
Roots, tubers, and stalks	12	0.35	9.2	2.1
Fresh fruits, excluding blueberries	25	0.18	10.7	2.0
Fresh fruits, including blueberries	26	0.18	44.4	3.7
Non-leafy vegetables	5	0.82	2.4	1.5
Animal tissue	13	0.078	3.8	1.0
Poultry and poultry products	6	0.30	1.1	0.50
Dairy products	7	0.028	1.6	0.47
Fish and sea foods, excluding oysters	6	0.12	0.39	0.25
Fish and sea foods, including oysters	7	0.12	2.2	0.51

Since the first three classes of foodstuffs listed in Table II, nuts, cereals, and dried legume seeds, are mainly dry matter, the manganese content is found to be correspondingly high. Leafy vegetables, which rank fourth in a comparison of materials in their native state, easily lead if the comparison is made on the dry basis. Since blueberries contain such an enormous amount of the element, the average for fresh fruits has been given both with and without the blueberry value. The average exclusive of blueberries is much more nearly representative of this class of foodstuffs and ranks the group in seventh place. The last three groups, poultry and poultry products, dairy products, and fish and sea foods are seen to be very low in manganese; all average about 0.5 mg. per kilo. of fresh material. In the group of fish and sea foods is found one material, oysters, which is much higher in manganese than the other members of the group. The average

manganese concentration of animal tissue (1 mg. per kg.) is doubtless greater than the average for meat actually consumed in the human diet since liver, a material containing a relatively large amount of the element, represents almost one-fourth of the samples analyzed, whereas it represents a much lower proportion of the meat consumed by an individual.

Variations within a given group were very great in many instances. The greatest regular variation was exhibited by the cereals and their products, in which group the maximum concentration (wheat bran) was 186 times as great as the minimum (corn flakes). Non-leafy vegetables were most uniform, with only a three-fold variation.

From the point of view of practical nutrition, the question arises as to what can or does supply the greatest share of manganese in the diet. Although nuts contain the highest concentration of the element, the intake of these is so low as to make them of little importance as sources of manganese for man. On the other hand, since the cereals and their products form such a large portion of the human diet, and since they also have a high concentration of the element, they probably contribute most to the supply of manganese in the diet. If calculated on the basis of food materials served in typical American diets as given by Rose (26, 27), the cereals and their products contribute about 38 per cent of the total manganese in two representative family menus. On the same basis of calculation, fruits and vegetables furnish 32 and 14 per cent respectively.

Only when the specific function or functions of manganese in the animal body have been uncovered and the amount needed daily for proper nutrition ascertained, can it be said that a given diet is adequate or deficient in this element. In view of the fairly high concentration of the element in cereals and their products as well as in fruits and vegetables, it appears unlikely that ill effects from inadequate amounts of manganese will result so long as these classes of food materials contribute their present share to the total food intake.

Summary

The manganese content of 83 representative food materials is given. The concentrations range from 0.028 mg. per liter of milk to 49.9 mg. per kilo. of bran flakes. Thirty-six of the materials contained less than 1 mg., twenty-five from 1 to 5 mg., and only twenty-two more than 5 mg. per kilo. of fresh material.

Arranged in descending order with respect to their manganese content, 12 classes of foods, representing 138 food materials, appear as follows: nuts; cereals and their products; dried legume seeds; green leafy vege-

tables; dried fruits; roots, tubers, and stalks; fresh fruits; non-leafy vegetables; animal tissue; poultry and poultry products; dairy products; and fish and sea foods.

Marked variations in manganese content were found among the several members of a given group.

In typical American diets cereals and their products contribute the largest proportion of the manganese intake.

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Editorial Review*

PHENOMENA OF RETARDED GROWTH

THE phenomenon of growth has long aroused the interest of investigators. The ordered accretion of living material, from the fertilized egg to the adult form has been considered a constant, a fundamental point of departure, for the studies of the biologist and, latterly, of the biochemist. This increase in the number of cells, in the size of the cells and in the material produced by the cells and deposited between them in the tissues is not, under ordinary circumstances, an irregular and uncontrolled activity. It normally takes place in certain more or less fixed intervals of time and the development of the various parts of the organism bears a definite relationship each to the other as well as to the growth of the organism as a whole. Furthermore, there are limitations upon the final size attained which are imposed by the germ plasm and which emphasize the fact that the phenomenon is characteristic of the species concerned. Heredity is, therefore, a primary factor governing growth and all growth therefore is, in a sense, restricted. However, in the present discussion the term "retarded growth" implies a limitation of development far more stringent than that imposed by the inherited tendencies of the organism.

Another factor governing growth is environment. While light, temperature and osmotic pressure may be of considerable importance in affecting development, the nature of the food materials is of great significance in furthering the optimal growth of which the species concerned is capable. Investigation in the field of nutrition has sought to evaluate the food requirements of the organism from both the qualitative and the quantitative point of view, for growth, maintenance, reproduction and in various abnormal conditions. In the course of some of these studies it has been observed that the development of experimental animals can be changed markedly by adjustment of the diet. The most convenient index of altered rate of growth is, of course, some measurement which can be made on the live, intact animal. It is natural, therefore, that observations on accelerated or arrested development have usually been based on changes in body weight or body size. While such criteria have yielded information of great value it is of interest to determine, if possible, other concomitants of altered rate of growth, such as structural changes not detectable in the intact

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organism, chemical variations in the tissues and body fluids and accompanying functional changes. Furthermore, in consideration of the various pathways taken by the digestion products of the common food principles in the course of metabolism, there seems little reason to expect that retardation of growth by the various devices thus far employed should always influence the development of the different parts of the body or their interrelationship in the same manner. This point of view has been justified by some of the older as well as by the more recent investigations in this phase of the field of nutrition.

Methods of Retarding Growth: Retardation of growth as measured by cessation of normal increase in body weight can be produced by various types of dietary adjustment. Strict limitation of the quantity of food given to an experimental animal will inevitably result in restriction of growth in proportion to the requirement of energy of the animal at that time. The young, developing organism has definite demands for food fuel and one of the early devices used to inhibit increase in weight was the restriction of the energy intake. However, with the growing conviction that certain constituents of the ration are indispensable for normal functions of the organism, there developed the realization that the gross restriction of food intake probably results in a deficiency condition which is exceedingly complex. When an unduly small quantity of a given food is allowed, not only is the energy ingestion curtailed, but the intake of the requisite vitamins, mineral salts and protein is likewise decreased. To eliminate the possibility of an actual shortage of such dietary essentials, the percentage composition of the experimental "low-calorie" diet is best adjusted so that the absolute daily intake of the indispensable components remains adequate in spite of the fact that the energy consumption is reduced (Winters, Smith and Mendel, 1927).

Limitation of ingested water is a possible method of retarding the development of the young animal, providing the experiment is of long enough duration. This is an especially severe restriction not only because water is an absolute necessity for the functions of the organism, but also because the young animal is normally more hydrated than the adult. Information of considerable fundamental importance could be elicited from such a study if the situation were not complicated by other dietary deficiencies. It has been demonstrated (Kudo, 1921; Jackson and Smith, 1931) that by limiting the intake of water the body weight of young rats can be maintained at a stationary level for a considerable portion of the life span of the animal. Also, deprivation of water is accompanied by a definite decrease in food consumption. The results of an experiment of this type must, there-

fore, be interpreted in the light of a deficiency of energy, water and, unless the ration has been suitably adjusted, of other indispensable factors. In the study of Jackson and Smith (1931) a group of animals consuming only as much food as was eaten by the test rats but receiving water *ad libitum*, served as a satisfactory control upon the factor of inanition. Under these conditions, effects specifically due to water deprivation were demonstrated.

Another method which has been used to retard the growth of young animals consists of restricting the quantity of protein consumed (Osborne and Mendel, 1915, 1916; Winters, Smith and Mendel, 1927). An experimental animal deprived of protein soon exhibits a complete loss of appetite. By regulating the quantity of protein in the diet, the rate of growth of the organism can, within limits, be controlled. This device will produce an uncomplicated "total nitrogen" deficiency only when the protein employed yields upon hydrolysis a complete series of the recognized essential amino acids in adequate quantity. Proteins which fail to yield requisite quantities of these indispensable amino acids will not satisfy the demands of the animal, in spite of the fact that the total amount of nitrogen provided would ordinarily be adequate. The amino acids whose indispensability for maintenance and growth rests on reasonably satisfactory experimental evidence are lysine, cystine, tryptophane and histidine (Osborne and Mendel, 1914; Rose and Cox, 1924; Johns and Finks, 1920); less convincing proof has been brought forward to indicate that the cyclic amino acids phenylalanine, tyrosine and proline are essential to physiological well being. It appears, then, that an experimental ration lacking or containing a limiting amount of an essential amino acid can serve to retard growth owing to a very specific dietary deficiency. The proteins gliadin from wheat and hordein from barley, both yielding on hydrolysis proportions of lysine too small to permit growth, have been employed extensively as the nitrogenous component of rations designed to maintain constant body weight of experimental animals without allowing growth.

Restriction of growth has been produced by feeding diets from which the inorganic salts have been removed to a large extent (Winters, Smith and Mendel, 1927; Smith and Swanson, 1929). While there is a moderate demand throughout life for minerals salts, this requirement is markedly accentuated in the young, growing mammal where tissues, notably those of the skeletal system, are being built up. The fact that in experimental animals restricted to such rations, the bones provide a readily available reservoir of certain highly important inorganic salts, renders the task of retarding growth by limiting the mineral elements of the diet extremely difficult. The problem of preparing a ration which is to be adequate in

all other respects than in its content of mineral salts, lies not with the organic components relied upon to yield the energy but with certain of the vitamin supplements. These consist usually of relatively impure substances with a considerable content of inorganic material. It thus happens that while one dietary essential is being incorporated in the food, the factor designed to be eliminated is included as a contaminant. As a result, students of the effect of limitation of mineral salts must employ a combination of vitamin supplements specially selected for their small content of inorganic salts.

It appears that the restriction of any constituent known to be indispensable in the diet is followed by interference with growth in the young and by difficulty of maintenance in adult animals. The lack of vitamins in the ration results in the appearance of changes which, because of the regularity with which they occur under prescribed conditions, have been considered as characteristic of lack of these food factors. The differentiation between the vitamins rests to a large extent upon the specificity of the changes produced by the absence of these adjuvants in the diet. Growth is inhibited in the absence of any of the recognized vitamins although doubt may reasonably be expressed as to whether or not this regularly observed cessation of growth is a primary or a secondary effect of the deficiency. While the limitation of vitamins in the ration is a device which can be depended upon to retard somatic development, the condition thus produced is an exceedingly complex one; there always occur accompanying structural and functional disturbances not apparent when, for instance, growth is restricted by underfeeding with a qualitatively adequate ration. It therefore follows that the classical symptoms of severe vitamin deficiency can scarcely be considered as phenomena of restricted growth alone.

The normal activity of the living cell is conditioned not only by the nature and amount of food materials brought to it but also by the efficiency of removal of the waste products of metabolism. In single-celled organisms excretion can only with difficulty be disturbed. On the other hand, in the higher animals incompetence of the apparatus for removal of waste products frequently exerts an untoward effect upon the well being of the animal. Evidence bearing upon this point was obtained from our studies of the growth of young rats after unilateral nephrectomy (Smith and Jones, 1927). A ration containing an extremely high concentration of protein retarded the growth of these animals whereas development was normal on a diet containing an ordinary amount of protein. It was suggested that the accumulation of nitrogenous metabolites exerted a generalized depres-

sing effect upon the organism which was reflected in the lowered food consumption and hence, in the growth. The high level of urea in the blood of these animals contrasted with that in intact animals on the same ration, supports this view.

Resumption of Growth: One of the phenomenal features of retarded growth is the retention, on the part of the experimental animal, of the ability to resume increase in body weight when the nutritive conditions once more become favorable. The consensus of opinion of many of the older investigators of problems of growth and nutrition was that the ability of the organism to increase in mass in the typical manner depended upon the exercise of this function during the portion of the life span in which it ordinarily takes place. In other words, it was assumed that unless growth occurs at the appropriate age, the ability to grow is, to a greater or less extent, lost. Experiments of Aron (1911) in which young dogs failed to regain the normal weight when they were realimented after long periods of maintenance at a markedly subnormal weight by underfeeding, favored this view. Studies with rats (Aron, 1914) likewise maintained on rations poor in protein, and experiments with very young rats which were underfed (Jackson and Stewart, 1920) showed a similar tendency toward the inability to regain the normal weight. On the other hand, the extensive investigations of Osborne and Mendel (1912, 1914 and 1915) indicate that the ability of the albino rat to resume growth is retained throughout prolonged periods of retarded development under the conditions of their experiments.

These investigators employed various devices for restricting the growth of their animals. Inadequate concentration of complete protein, deficiency of the essential amino acid lysine, insufficiency of energy and limitation of the amount of vitamins apparently produced, in general, similar results as concerns the maintenance of body weight and the subsequent ability to grow. Neither the body weight at which the maintenance was begun nor the duration of the stunting period had an influence upon the latent power to grow. Repeated instances of restricted development extending well beyond the age at which growth normally ceases are recorded in these studies; when the ration was made adequate growth was resumed and in many cases a body weight normal for the age ultimately reached. For instance, a female rat weighing 52 grams at 35 days was permitted to grow at a subnormal rate so that at 303 days of age she weighed only 73 grams. Upon realimentation, this rat grew at an accelerated rate until she weighed 376 grams 180 days later. The ability to reproduce was not lost in these animals and the milk of the mother provided for normal growth of the progeny during the period of lactation, although she herself was prevented

from growing by the defective dietary regime. These young grew at the usual rate when provided with satisfactory food after weaning. Not only is the very ability to resume growth remarkable, but the fact that the normal body weight for age of these previously retarded animals is frequently attained in a shorter time than usual, is a phenomenon worthy of note.

The persistence of the ability to grow has been shown in another way by studies (Smith and Moise, 1924) in which the rate of regeneration of injured liver tissue was measured in rats subsisting upon a ration deficient in lysine, the body weight of these young animals being thus maintained at a constant level. It was observed that, after a standard degree of hepatic necrosis induced by chloroform, the repair of the liver proceeded as rapidly in these stunted rats as in the control animals consuming an adequate ration. Here is evidence of the conservation of available essential material for reconstruction of a damaged vital organ at the same time that general somatic growth is not possible. Furthermore, it indicates that the chemical processes involved in repair may not be the same as those of growth.

This phenomenon of resumption of growth may be looked upon as a defense mechanism. The restriction of development resulting from adverse environmental conditions is automatically compensated by subsequent growth at an accelerated rate. Indeed it has been shown that not even under ideal conditions of nutrition does growth take place with complete uniformity. There are cycles of acceleration followed by periods of retardation in normal growth curves of animals and plants. These irregularities have been studied in man, fowl, rat, guinea pig, cow and mouse, as well as in plants and unicellular organisms (Robertson, 1923; Brody, 1928). The studies upon laboratory animals have indicated the surprising extent to which growth may be depressed without exceeding the limit of possibility of resumption of growth after favorable conditions are again established.

Change in Body Form: The change in conformation of the body is one of the early recognized and very constant accompaniments of maintenance of body weight at a subnormal level. One of the first series of systematic observations of this phenomenon was made by Waters (1908, 1909). In his studies a large number of young steers was underfed for varying periods up to a year in such a way that there was no increase in body weight. The form of the stunted animals was distinctly different from that of the control group; the length of the fore leg had increased and hence, the height at the withers, the length of the body was greater than at the beginning of the experiment, and the head became longer. There was also a dorsoventral elongation of the thorax. These changes took place at the expense

of adipose and muscular tissue and at a rate only moderately below normal. The resulting animals were emaciated, and obviously too tall and too long for their body weight. Similar observations were made by Aron (1911) upon puppies held at nearly constant weight by the limitation of their energy intake. In this case there was almost a normal increase in both length and height with a resulting distortion of bodily contour. It is unfortunate that in neither of the above-cited series of experiments were the stunted animals compared to normals of the same body weight, a comparison which brings out the alterations in body form much more clearly.

Somewhat similar changes in form have been reported in rats maintained at subnormal weights by limitation of water. Marked increase in length of tail and in weight of skeleton were observed under these conditions by Kudo (1921) though body length was little affected. These studies were of relatively short duration and it is uncertain whether water was the only deficiency in the diet. In experiments somewhat better controlled but with too few animals, Jackson and Smith (1930) showed an increase in body length: body weight ratio in rats maintained at a markedly subnormal weight by limitation of water intake. That a deficiency in vitamins results in a generally similar change in shape of the body has been shown by Quinn, King, and Dimit (1929). Quantities of vitamin A so small as to permit growth of only 22 to 30 grams in 8 weeks were given to very young rats with the result that at the end of this time there was evidence that the body length, leg length, chest girth and width of hips had increased to a relatively greater extent than had the body weight. Similar distortions of body form were detected in rats maintained at a constant weight through limitation of vitamin B (complex) by these investigators.

An attempt to differentiate between the structural effects of inhibition of growth by various methods was made by Winters, Smith, and Mendel (1927). Male rats weighing 40 to 50 grams were maintained for 40 days at that weight by giving one of the following deficient rations: low-calorie, low-protein, low-lysine and low-salt. Except for the deficiency indicated, the diets were adequate. At the end of the experimental period (40 days) it was found that, compared to normally growing rats of the same weight, the animals on each of the above diets had increased in body length although the weight had remained constant. This elongation was greatest in the group given the low-lysine food (8 per cent) and least with the animals receiving the diet poor in salts (3 per cent) although in every case the difference in length between the normal control and the stunted rats was statistically significant. It was also shown that the tails of the stunted rats were too long, most pronounced in the case of the low-salt animals.

The humeri, ulnae, radii, femora and tibiae were measured; these bones were 10 to 20 per cent longer than those of normal rats of the same weight. Comparing the expected growth with that obtained, it was found that during retarded development under these conditions, the stunted animals made from 9 to 20 per cent of the expected gain in body length and 36 to 40 per cent in length of leg bones. All groups of the stunted rats in these studies showed a slightly greater than normal size of cranium and length of mandible.

There is little room for doubt, on the basis of the available literature, that, whereas it is possible to retard the characteristic increase in body weight by a variety of methods involving an adjustment of the ration, little can be done to prevent the persistent growth of the bones. Thus, in spite of a stationary body weight, the physique of the experimental animal slowly changes, the length and height of the body increasing and the thoracic cavity becoming deeper and narrower. These alterations of body form are usually accentuated by the shift of material from the adipose and muscular tissue. It appears, furthermore, that under closely comparable experimental conditions the degree of structural change resulting from this persistent impulse to grow is dependent, to some extent, upon the nature of the deficiency in the ration.

Weight and Composition of Bones: Since growth is looked upon as a correlated increase of parts of the body, it might be expected that restriction of development of the organism as a whole would result in a proportional retardation of all the constituent parts. However, the facts already noted with reference to the linear increase of the long bones throw doubt upon the validity of such an assumption. One of the constant accompaniments of retarded growth is the increase in fresh weight of the bones. This was brought out in a striking manner by the study of Aron (1911) on two dogs from the same litter, one of which was permitted to grow and the other prevented from increasing in weight. At the end of 200 days the first animal weighed 5.8 kilos while the second weighed 2.85 kilos. In spite of this great discrepancy in body weight, the fresh weight of the bones of the stunted animal was only 6 per cent less than that of the well-fed dog. Restriction of water intake to the extent of retarding growth results in a definite increase in the weight of the bones (Kudo, 1921; Jackson and Smith, 1931). The various types of stunting employed by Winters, Smith, and Mendel (1927) resulted in increases in the dry weight of the bones up to 90 per cent of that of control animals of the same weight, the greatest accretion of weight taking place in the group of rats retarded by a deficient intake of energy ("low-calorie") and the least in the group on a diet poor

in mineral salts. All of the long bones in the leg were used in these determinations and, except in the "low-salt" group, each bone showed an increase in weight.

It thus becomes apparent that in the elongation of the bones brought about by the restriction of growth, there occurs concomitantly an increase in the weight of the bones, *i.e.*, the proportions of the individual bones remain more or less normal. Quinn, King, and Dimit (1929) found that with the possible exception of the humerus, rats whose growth was retarded through a lack of vitamin A had leg bones which, though too long for the body weight, were of normal proportions. In their group of animals upon a limited vitamin B intake the proportions of the humerus were abnormal though all the other leg bones measured showed the expected relationships between length and thickness. It therefore follows that the persistent increase in the weight of the bones, often in the normal proportions if not to the normal degree, takes place at the expense of other tissue constituents, the gross body weight being stationary, and that changes in composition of the bones might be expected under conditions of retarded growth.

A marked change in composition of the bones of his underfed dogs was observed by Aron (1911). In one experiment the bones of the stunted animal had approximately the same length and weight as those of a dog weighing twice as much. Upon analysis, however, it was found that the bones of the restricted animal had 17 per cent more water than those of the normal dog and that the fat had entirely disappeared, water in the starved bone taking the place of fat in the normal structure. On the other hand, there was only a slight reduction in the percentage of protein, and the ash content remained unchanged. These observations on simple underfeeding are of interest in the light of those (Smith and Schultz, 1930) with young rats maintained at constant body weight by strict limitation of inorganic salts. These animals were compared to normal rats of the same weight and to others of the same age. The fresh weight of the femora was greater than that of normal rats of the same body weight but less than that of controls of the same age. Growth of the bones had persisted in spite of the pronounced lack of the characteristic building material for this structure. The water content of the "low-salt" bones was much greater (16 per cent) than that of animals of the same age but somewhat less than that of the weight controls. The relative amounts of ash and fat in the bones of the stunted rats were the same as those of rats of the same body weight but when the alcohol-ether-insoluble organic residue of the "low-salt" bone was compared with the weight controls a marked difference in favor of the "low-salt" bones was apparent; indeed, the concentration was slightly greater

than that of normal rats which had grown to more than three times the body weight of the "low-salt" animals. The normal growth of bone in the rat is characterized by an increase in the percentage of organic residue (Hammett, 1925); the striking increase of this constituent in the rats upon the salt-poor ration affords still further confirmation of the persistence of the growth impulse in bone of animals in which increase of body weight is prevented. These studies, to be sure, represent a more specialized condition than in the case of gross underfeeding for here one of the specific bone constituents is lacking, in large measure, from the diet. The increased deposition of organic residue, the prepared pattern for real bone, offers a possible explanation for the accelerated development observed in these animals, once the ration is made adequate.[1]

Changes in Organs: Is it likely that other organs show, like the bone, a disproportionate change in size under conditions of restricted growth? It is especially reasonable to expect such changes because of the essential localization in certain organs of the metabolism of given food principles concerned in the stunting. Just as the deposition of salts in the bone is adversely affected by the consumption of rations extremely poor in salts, so the liver and kidneys may be influenced by adjustment of the dietary protein because these organs are especially involved in the deaminization of amino acids, the disposal of the non-nitrogenous rest, the synthesis of urea and finally, the excretion of the nitrogenous products of metabolism.

With the exception of the brain, the organs of dogs, maintained at a markedly subnormal weight by underfeeding, retained their weights and thus their normal relation to the body weight (Aron, 1911). The brain of the stunted animals continued to grow so that at the end of the experimental period, it constituted 2 per cent of the body weight whereas in the normal animal it was 0.9 per cent. Hence it appears that the internal organs are called upon less than other tissues to contribute substance to make good the continued growth of the skeleton. The data also show that there was a complete loss of stored body fat and a very large diminution of fat in the organs. A marked loss of total body protein occurred; this arose primarily from the muscles. On the other hand, there was found an increase in protein in the bones, a means whereby some of the muscle protein was conserved to the body. The loss in muscle protein and body and marrow fat is made up by an increased proportion of water. This explains one of the characteristic observations in the study of inanition, namely, that in animals subjected to dietary management which does not permit

[1] Unpublished observation from this laboratory by Mr. R. O. Brooke.

growth, the calorie value per gram of tissue is definitely lower than that of normally fed individuals.

That the changes in the size of organs are not the same in all types of restriction of growth by diet was shown by Winter, Smith, and Mendel (1927). In rats whose body weight was maintained at 40 to 50 grams for 40 days by insufficient energy intake, uncomplicated with other deficiencies, the liver, heart and kidney had a normal weight for animals of that size whereas the testes were markedly enlarged. When the rats were stunted by a deficiency of protein, the kidney and testes were normal but the liver and heart were enlarged. When the proportion of lysine in the ration was unsuited for growth, the liver had a normal size for body weight but the heart, kidney and testes were much too large. Strict limitation of inorganic salts was followed by a persistent growth of liver, heart, testes and kidney, the enlargement of the kidney under these conditions being especially striking. It was subsequently shown (Smith and Schultz, 1930) that this increase in weight of the kidney is accompanied by an augmented content of ash in the organ; and by the usual decrease in moisture with increasing age. Smith and Swanson (1931) presented supplementary data on the kidneys of rats given the salt-poor ration for 90 days, the body weight being maintained at 150 grams. After 21 days on the experimental ration, the same renal enlargement was observed as in the previous studies and the water content was normal. The age of the rat at this time was the same as that of the animals studied by Smith and Schultz. After 42, 63 and 90 days on the experimental diet there was a slight regression in weight but an increase in moisture content so that after 90 days the kidney, still too large for the body weight, was definitely hydrated. These studies were carried out upon comparable groups of animals and under carefully controlled dietary conditions in the light of modern nutritional principles. Large enough numbers were used so that the differences noted are statistically reliable.

A still different combination of effects upon the organ weights is brought about by a restriction of water (Jackson and Smith, 1931). In these experiments the eyeballs, kidneys, ovaries, testes, hypophysis and suprarenals were enlarged; the brain, stomach and muscle were unchanged and the liver, spleen, skin, thymus and thyroid decreased. Accompanying the changes in size there was a dehydration, the skin losing most water and the brain least. It appears that, although growth can be retarded and body weight maintained for relatively long periods by various deficient rations, a closer study of the detailed structural response of the individual organs

of the body indicates differences which are more or less characteristic of the dietary adjustment causing them.

Effects upon the Blood: Jackson (1925) has emphasized the constancy of the composition of the blood during inanition. Apparently there are few changes in this body fluid or its components which are characteristic of inanition and restricted growth. In a study of the blood of albino rats whose growth was retarded by strict limitation of inorganic salts during the period of rapid development, Smith and Swanson (1929) demonstrated a unique type of polycythemia. The erythrocytes were subnormal in size and the cell volume of the blood was therefore low. There was a marked reduction in corpuscular hemoglobin which, in spite of the large number of cells, resulted in anemia. However, the blood volume was normal and the concentration not increased. This condition was shown to be due to lack of salts rather than to the voluntarily reduced energy intake of the experimental animals. The marked changes in the blood did not appear unless there was a definite cessation of growth. When a complete salt mixture was given, the high erythrocyte count returned to normal, the hemoglobin increased, the cell volume increased and the previously high reticulocyte count showed a tendency to decrease (Swanson, Schultz and Smith, 1930). These phenomena are thus distinctly correlated with the sole dietary deficiency involved—namely, the lack of inorganic salts. Apparently there is a close dependence of both body weight and of normal blood composition upon a certain minimal concentration of mineral salts in the ration.

Influence on Behavior: In addition to the structural changes thus far discussed, it is reasonable to expect that such striking alterations in size, proportion and composition of organs would be accompanied by demonstrable changes in function. The question of the behavior of experimental animals which have been severely stunted during the period of most rapid growth is one of considerable significance. Will such individuals react to situations in the same manner as normal animals of the same size and, therefore, much younger, or can certain of the elements in the behavior be attributed to processes whose full development depends on age? Again, is it possible to establish a correlation between behavior and a certain type of dietary deficiency to the extent that such a correlation has been demonstrated between dietary deficiency and structural changes? In general, as the result of uncontrolled observation, it has been concluded that a malnourished child is likely to be dull and lethargic. Lusk (1921) has described the mental apathy, muscular inertia and difficulty of sustained effort among the population in Germany during the World War. On the other hand, there is some evidence pointing to the fact that undernourished

school children are not mentally inferior to normal ones and may be somewhat superior (Hunt, Johnson, and Lincoln, 1921).

The behavior of stunted animals has been compared to that of normal controls by Anderson and Smith (1926). This preliminary investigation indicated that rats stunted by underfeeding and by the lack of lysine were superior to normal animals of the same age in relearning an old problem as well as in learning a new one. The measure of performance was the speed and accuracy shown in running a moderately difficult maze. In order to obtain more varied measures of performance as well as to control the effect of age in the experimental groups, a second and more extensive series of studies was carried out (Anderson and Smith, 1931).

A large number of male rats 24 days old were divided into 10 groups, each with the same mean body weight and dispersion. One group was tested immediately and served as the normal weight control for the stunted animals. Forty days later, a group which had grown normally from the beginning, a group which had been given only enough of an adequate diet to maintain body weight at 40 grams and a group stunted with the gliadin ration were given the behavior trials; at 80 days after the beginning of the experiment similar groups appropriately fed from the beginning were tested and finally at 120 days three more groups, similarly fed, were tested. The measures of performance used were the speed and accuracy of learning to run through a moderately difficult maze, voluntary activity in a revolving cage, the time required to traverse a simple habituation box without culs-de-sac, and the time required to open the exit door of a problem box. The experimental rations were so constituted that, so far as could be gauged by present standards of nutrition, there was only the single deficiency in each case.

In practically all of the devices employed to measure the behavior of the animals there is a difference between the controls and the restricted animals and, in some instances, between the two types of stunting. As measured by the results in the activity cage, the low-calorie rats are the most active group at every age tested, the low-lysine, less active and the normal controls least active. With advancing age, the activity displayed by a given nutritional group decreases. In the stunted groups, therefore, one sees the persistence of the activity of youth in the general level of performance, upon which is superimposed the age factor of which the progressive decrease in activity is the evidence. Increasing the length of the stunting period prior to testing does not increase the activity of the rats in either of the restricted groups. One of the striking observations made was that after realimenting and after the body weight of the previously stunted

animals had almost reached that of the control rats, all differences in activity between the groups vanished. In this measure of simple activity there is a difference between the effects of the two types of stunting employed, the gliadin-fed rats behaving at all ages measured, very nearly like normal rats of the same body weight.

Rats receiving an insufficient energy allowance for growth run a simple habituation box and a fairly complex maze in less time than either normal animals of the same age or those similarly restricted through a deficiency of lysine. Here again, in those measures of performance involving speed, there tends to be a slowing-up with advancing age in all groups. On the other hand, it was found that the low-calorie group made the greatest number of errors while running the mâze, the gliadin-fed rats somewhat fewer and the normal controls the least number. In other words, the stunted rats are extremely active but not accurate while the normal animals present the reverse behavior characteristics. Here again, as in the activity experiment, there is a differentiation on the basis of maze errors between the two restricted groups. Both the normal rats and those of the gliadin-fed groups become somewhat more accurate with advancing age; this is not true for the low-calorie groups. Strikingly enough, after the erratic, stunted rats have been realimented they and the normal controls exhibit a higher degree of accuracy than before but the previously stunted rats still make more errors than the controls, a fact suggesting that the error-making habit characteristic of their earlier trials has persisted in spite of the change in diet.

In solving the problem box situation the rats lacking lysine tend to be superior. In all groups the older rats are able to make a better record with this measure of performance than the younger ones. After realimentation all three groups solve the problem box better than they did before but the low-calorie group exhibits a persistence of inferiority. It appears, in general, that on the basis of age within groups, youth favors those measures depending upon speed while greater accuracy is shown by the older animals.

One of the uncertainties concerning the experimental animals whose growth has been retarded lies in the propriety of considering them as typical of normal individuals of the same size versus those of the same age. A very young rat maintained upon a ratio poor in lysine at its weaning weight of 45 grams for 40 days during which interval of time its litter-mate consuming an adequate ration has more than tripled its weight, resembles a normal animal of the size in weight only. The above discussion has pointed out the striking feature of continued growth of the skeletal system in all

types of retarded development thus far studied. Furthermore, certain of the organs change, not uniformly under all experimental conditions, to be sure, but regularly enough both in size and in composition to emphasize the fact that, in spite of a body weight maintained at a youthful or subnormal level, the animal is not like a young rat either from the structural or from the chemical point of view. A stunted rat bears little resemblance to the normal young individual of the same weight; the head and nose are somewhat longer, the body is elongated, the legs are longer and there is a typical gaunt appearance which becomes more pronounced the longer the duration of inhibited growth. On the other hand, the restricted animal is not like a normal rat of the same age either in proportion or in composition.

On the basis of the results of the behavior studies it is equally impossible to classify a stunted individual exclusively either with the youthful group or with the mature age controls. In general, in measures of behavior involving speed, the restricted animals tend to resemble the normals of the same body weight while in the problem box they approach more closely the age controls. In no sense can it be said that an animal whose growth has been severely retarded has retained entirely its youthful characteristics or has attained completely those typical of its age. Such an individual is essentially a different organism with a structure, composition, behavior and, possibly, a metabolism, more or less characteristic of this state of existence.

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STUDIES ON LACTATION*

I. PRODUCTION OF MILK IN THE DOG AS INFLUENCED BY DIFFERENT KINDS OF FOOD PROTEINS

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MOST of the experiments concerning the influence of diet upon lactation have been conducted for commercial purposes on cows and goats. These animals are the result of several centuries of intensive breeding, and the capacity of the mammary gland, and probably their entire metabolism, have been so specialized that we are not able to make direct comparisons of the findings in these animals with those on other animals and humans, where the process of milk secretion has not been so highly developed.

Ssubotin (1866), working with dogs, found that the kind of nourishment had a very important influence upon the composition of milk. Voit (1869), one of the earliest workers on the influence of diet upon the composition of milk, also experimenting with dogs, found that high protein feeding favorably influenced milk secretion. Although his individual experiments were not of long duration, they seem to have been complete and well controlled. The work of these two investigators, along with that of many others in their period, seems to show that the diet is an extremely important factor in determining the secretion of milk, and that the protein constituent of the diet greatly influences its composition.

Hoobler (1917) studied the effects upon human milk production of diets containing various forms and quantities of protein. The nutritive ratios varied from 1:4 to 1:15, and the different types of protein used were meat (round steak), milk, egg, cereal, and nuts. The diets of narrow nutritive ratio gave positive nitrogen balances and increases in the milk

* The material in this paper is taken from a thesis submitted to the University of Rochester in partial fulfillment of the requirements for the degree Doctor of Philosophy. The experimental work was done under a fellowship granted by the National Live Stock and Meat Board.

nitrogen, whereas the diets of wide nutritive ratio gave negative nitrogen balances and, as Hoobler states, the effect was that of "milking the flesh off their backs." It was hard to keep the mothers in positive nitrogen balance on vegetable protein, no matter how small the nutritive ratio. On the animal protein diets, the mothers stayed in positive nitrogen balance in ratios up to 1:6. When he calculated the efficiency of the diets, he found the greatest efficiency in a diet having a ratio of 1:4, and containing two-thirds or more animal protein. This work not only brings out the fact that relatively high animal protein diets are beneficial, but that the quality of the protein is of great importance.

One of the very few adverse reports in the literature is that of Hartwell (1922b) who found that large quantities of protein fed to a nursing rat are detrimental to the young even when the diet contains all of the essential constituents. In a lactating rat the amount of protein constituting an excess varies with the type of protein and with the individuality of the rat. Simmonds (1924) critically examined Hartwell's work and believed that her evidence was not conclusive enough to allow her to state that high protein diets are injurious. Simmonds herself was not able to produce any injurious effects when the protein constituted 67 per cent of the diet. Hartwell (1924) later on, in her study of the effect of protein excess in a lactating rat's diet, came to the following conclusions: "It is impossible to state what constitutes an excess of protein in a lactating rat's diet, because there is a quantitative relation between protein and vitamin B (complex). Thus, provided the vitamin B is increased when the protein is increased, there should be no evil effects. In the lactating rat the function of vitamin B is primarily concerned with the metabolism of protein *qua* protein or with the nitrogen part of the molecule; for to a diet of 15 grams of bread and 5 grams of protein must be added far more vitamin B than to a diet of 15 grams of bread and 5 grams of starch. A high proportion of protein (15 grams of bread to 21 grams of food casein) in the lactating rat's diet produces no harmful effect in the offspring, provided the diet is very rich in vitamin B, but the rate of growth of the sucklings is impaired."

If such a relation be true, it may well account for some of the rather slight results obtained by feeding high quality proteins. The good effects of the protein may have been masked by an insufficient supply of vitamin B.

Watson (1907), observing the influence of an excess of meat diet upon lactation in rats, states that a meat diet affects prejudicially their powers of reproduction and lactation, and in the article he suggests that the increase in consumption of animal food in England may be an important

factor in the causation of the decreased birth rate, and the diminished powers of lactation. On the other hand Hitchcock (1926) has shown that female rats that were nursing litters weighed more and raised larger and healthier litters, whose growth rate was faster, when they were fed meat in addition to an adequate balanced diet.

Sherman and Muhlfeld (1922) fed breeding rats upon diets containing respectively one-sixth whole milk powder to five-sixths whole wheat, and one-third whole milk powder to two-thirds whole wheat. Young were reared on both diets, and both diets can be regarded as adequate for growth, reproduction and successful suckling of the second generation. The larger proportion of milk in the second diet resulted in an improved nutrition for the lactating mothers. Here again the fact that animal proteins are better suited to the synthesis of milk than are vegetable proteins is brought out. Sherman and Campbell (1924) also found an increase in the milk constituents of the diet to give evidence of an improved nutrition in the lactating rat mother. Cary and Meigs (1924) cite the work of Morgen and his collaborators who showed that changes in the dietary protein affected the milk yield through a wide range in the level of the protein feeding. Cary and Meigs' own work shows that milk secretion in cows is markedly affected by changes in the dietary protein.

Hart and Humphrey (1919) state that the only safe procedure for the maintenance of high milk production in cows is through the use of a high plane of protein intake which, although of low relative efficiency, should be drawn from the plant protein concentrates rather than from those of animal origin. It is only natural to suppose that an herbivorous animal should derive its necessary protein material from a plant source rather than from an animal source.

In searching for some specific protein responsible for lactation, Hartwell (1922a) states that her experiments suggest that edestin is a very good protein, and that it improves the growth curve of suckling rats when the mother's diet is deficient in protein, but, when added to a diet containing an adequate amount of protein, it has no effect.

Adair (1925) has obtained significant results in connection with human mothers. He observed 400 cases in which he studied the weight changes in the babies for twelve days after birth. The babies were weighed before and after feeding to determine the amount of milk taken from the mother. The efficiency of breast feeding was determined by the effects on the offspring. He placed a hundred mothers on each of the following diets; high protein, high carbohydrate, high fat, and balanced. The following table shows the intake ratio of one diet to another.

The high protein diet and the hospital diet showed the highest progressive increase in the amount of milk secreted. Excess feeding had no stimulating effect upon quantitative secretion. The high caloric diet caused an increase in the mothers' weight, which was associated with decreased milk secretion. He therefore concluded from this work and later observations that liberal amounts of protein in the diet are desirable for human lactation.

TABLE I

Diet	High protein		(Hospital) balanced		High carbohydrate		High fat
Calories	23	:	30	:	32	:	39
Protein	42	:	31	:	31	:	33
Fat	85	:	104	:	132	:	207
Carbohydrate	107	:	141	:	189	:	152

EXPERIMENTAL

From the foregoing account of the literature we learn that milk production is definitely altered by changes in the quality and the quantity of the protein in the diet. Fairly large amounts of animal protein increased lactation in humans, rats and dogs. The questions that now arise are, can diet definitely affect the quality and quantity of milk produced over the entire lactation period; and what is the best source of protein available for lactation?

Cary and Meigs (1924) believe that the changes in the dietary protein affect milk secretion largely by inducing changes in the quantity and the quality of the amino acid mixture circulating in the blood plasma. Therefore, if we insure a good quality of amino acids in the blood, we should be able to stimulate the mammary gland to its maximum efficiency. Milk, to insure the best nourishment for the young, must contain a complete protein. Since the immediate precursors of the milk constituents are in the blood, by feeding a complete protein we should be able to produce the same in the milk. As has been shown by Hart and Humphrey (1915), milk is one of these proteins. Because there is this direct relationship between the dietary constituents and those of the milk, it is only natural that milk in the diet would be an excellent source of protein. But this does not exactly solve the question of what proteins stimulate milk secretion.

The liver is an organ that probably has many more functions than those already discovered. Therefore it is probably more significant as a food

than many other animal proteins. It has been studied in connection with blood regeneration by Whipple and his collaborators and many others. Since milk production seems to be intimately linked with the quality of the blood, it might be expected that liver would be an excellent source of protein for milk secretion.

Kidney is also a glandular organ of the body and has been shown by Mitchell and Beadles (1926, 1927) and Hoagland and Snider (1926) to have the same biological value as liver. Kidney may also be of some importance in milk secretion.

Egg has been shown by Mitchell and Carman (1924) to have a biological value, as estimated by nitrogen balance studies with rats, of 93 as compared with 77 for both liver and kidney (1926, 1927). This, along with the fact that eggs as well as milk are products of reproductive changes, would indicate that egg protein might be influential in stimulating milk production.

In order to study the effects of these various proteins, it was thought best to use the dog as the experimental animal. Boston bull dogs were chosen because their size makes them small consumers of food, they are of a quiet and friendly disposition, they are clean because of their short hair, and they are good milk producers. For the most part pedigreed stock was used in an attempt to control as far as possible the hereditary factor which, no doubt, plays a very important part in the production of milk. It was planned to use dogs of as nearly the same size and age as possible, to breed the females for the most part to the same sire, and to keep the habits of life as regular as possible, making sure that one dog received no more exercise than another. During pregnancy the mothers were allowed the freedom of the room for about one hour a day. The remainder of the time they were confined to metal dog cages, with the exception of a few short walks in the open air. In all cases the litters were limited to three pups, two male and one female. These dogs generally have five or six pups to a litter, but it was thought that by limiting the litter to three, we would allow for any casualties at birth, and also be assured that the mother would not be over-taxed at any particular period of lactation. The experiments justified this limitation for in all cases the three pups alloted to the mother were well able to take care of all of the milk produced. The pups were chosen at birth, so that their total weights would conform as closely as possible to that of the pups of earlier experiments.

Criteria of milk production. Many methods, both direct and indirect, have been used to determine the quality and quantity of milk produced by mammals. Direct quantitative collection and chemical analysis is the

method employed with such as dairy cattle, where one has no trouble in obtaining large quantities of milk. But where one is dealing with smaller mammals, it is often very difficult to obtain enough milk for analysis, and one must resort to the rate of growth of the offspring. In humans the milk production is often judged by weighing the infant before and after each feeding, thus permitting the natural emptying of the breast and also allowing the infant directly and naturally to benefit by the milk produced.

Dogs were chosen for this study partly because it would be possible to apply both direct and indirect methods. The growth changes in the pups were studied over the period of the first four weeks of lactation, and then the pups having been separated from the mother during the fifth week, the milk was collected quantitatively by manual manipulation four times a day, care being taken that the glands were drained completely at every milking. The milk of each milking was placed in a small stoppered bottle and put in the ice box. At the end of the day these were pooled and the day's milk analyzed. The constituents varied very little from day to day over this short period. The marked daily variations that have been noted in cows were not apparent in these experiments with dogs.

Another important criterion of the efficiency of a lactation diet is the nutritive condition of the mother. Therefore metabolic studies were done during the ninth week of pregnancy, third week of lactation, fifth week of lactation and sexual rest. The blood as well as the urinary chemistry was studied in an attempt to note any physiological changes that might occur during these periods.

Methods of analysis. Carmine was fed as a marker for the fecal excretion at these periods before the morning meal. The mothers were catheterized and blood was drawn, also before the meal, to insure basal nutritive level. The mothers ate all of the food with few exceptions, sometimes requiring some coaxing or pampering on the part of the feeder. The urine was collected in 1000 cc. flasks containing a few drops of toluene (for preservative), and analyzed for total nitrogen by the Kjeldahl method; ammonia-nitrogen, Folin (1925) method; amino acid nitrogen, Henriques-Sörensen (1910) formol titration method; total sulfur, Benedict's (1909) method; total phosphorus, Fiske and Subbarow (1925).

The feces were collected in a covered jar of 95 per cent alcohol, dehydrated to dryness by heating on a steam bath after several applications of 95 per cent alcohol, pulverized, and analysed for total nitrogen by the Kjeldahl method. Blood analyses were done immediately upon collection. Approximately 7 cc. of the blood were centrifuged in a graduated centrifuge tube, thus determining the volume per cent of the corpuscles and plasma. Two cc. of the plasma were used for blood lipid determination by Bloor's (1928) method. Five cc. of the fresh blood were used in the preparation of Folin-Wu blood filtrate (Haden's modification, 1923). The following determinations were made upon the filtrate: blood sugar, Benedict (1926) method; non-protein nitrogen, micro-Kjeldahl method (Folin and Wu, 1919); and amino acid nitrogen, Folin (1922) method.

The milk was analysed for specific gravity by means of a 10 cc. specific gravity bottle; fat by the Babcock method; total proteins by the Kjeldahl method; casein and albumin by coagulation,

and the Kjeldahl method; total solids and ash by the usual methods; and lactose by Benedict's (1911) method.

Diets. The mother was started on the experimental diet approximately one week after conception, and continued on this diet through the fifth week of lactation. The diets were fed during pregnancy in order to be sure that the dog's metabolism had definitely adjusted itself to the experimental diet, before studying the effects of such a diet upon lactation, and to insure the same nutritive foreground for the development of the mammary glands, as was used in the study of their milk production. This procedure may be partially fallacious in that the results may also include the effect of the diet upon the intrauterine life of the pups. However, we feel that this procedure is better than one which does not provide for a similar nutritive foreperiod.

The diets were constructed according to Cowgill's suggestion (1923). He suggests a caloric intake of from 70 to 80 calories per kilo. per day; 0.8 gram of nitrogen per kilo.; 25 per cent by weight of fat; 0.4 gram bone ash; 0.2 gram salt mixture. This allows a nutritive ratio of from 1:3.1 to 1:3.5, thus permitting a liberal supply of protein.

The diets were as nearly identical as possible except for the source of protein. They were constructed upon a kilo. basis according to the plan shown in Table II.

TABLE II

0.7 to 0.8 gram of nitrogen per kilo	40% carbohydrate 2 to 3% variation
75 to 80 calories per kilo. (4–9–4 basis)	6% ash 2 to 3% variation
30% protein 2 to 3% variation	Nutritive ratio of 1:3.1 to 1:3.5.
25% fat 2 to 3% variation	

Tables III, IV, V, VI and VII show the details of the diets used. All quantities are in grams per kilo.

TABLE III

Diet No. 1

Food	Total	Protein	Fat	Carbohydrate	Water	Ash
Egg	9.00	3.937	3.821	—	0.810	0.3726
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	—	—	—	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	19.77	4.168	3.976	5.937	4.584	1.0336
−water	4.584					
Solid weight	15.186					
% by weight	—	27.4	26.1	39.1	—	6.8
Calories per kilo. total of 77	—	17	36	24	—	—

Nutritive ratio 1:3.5 .666 grams nitrogen per kilo.

TABLE IV

Diet No. 2						
Food	Total	Protein	Fat	Carbo-hydrate	Water	Ash
Liver	22.20	4.528	0.999	0.377	15.806	0.3552
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.70	—	2.700	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	35.67	4.759	3.865	6.134	19.580	1.0162
—water	19.58					
Solid weight	16.09					
% by weight	—	29.5	24.0	39.2	—	6.3
Calories per kilo. total of 79	—	19	35	25	—	—

Nutritive ratio 1:3.1 .761 grams nitrogen per kilo.

TABLE V

Diet No. 2a						
Food	Total	Protein	Fat	Carbo-hydrate	Water	Ash
Liver	19.30	3.937	0.868	0.327	13.735	0.3086
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.94	—	2.942	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.464	—	—	—	—	0.4640
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato juice	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	33.074	4.168	3.976	6.264	17.509	1.0336
−water	17.509					
Solid weight	15.565					
% by weight	—	26.7	25.5	40.2	—	6.6
Calories per kilo. total of 78	—	17	36	25	—	—

Nutritive ratio 1:3.6 .666 grams nitrogen per kilo.

TABLE VI

Diet No. 3

Food	Total	Protein	Fat	Carbo-hydrate	Water	Ash
Round steak	21.67	4.530	2.297	—	14.692	0.2167
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	1.402	—	1.402	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt mixt.	0.20	—	—	—	—	0.2000
Tomato	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	33.842	4.761	3.865	5.837	18.466	0.8777
—water	18.466					
Solid weight	15.376					
% by weight	—	30.8	25.1	37.9	—	5.7
Calories per kilo. total of 78	—	19	35	24	—	—

Nutritive ratio 1:3.1 .761 grams nitrogen per kilo.

TABLE VII

Diet No. 4

Food	Total	Protein	Fat	Carbo-hydrate	Water	Ash
Kidney	27.31	4.533	1.310	0.109	20.946	0.3277
Sucrose	1.89	—	—	1.890	—	—
Corn starch	3.78	—	—	3.780	—	—
Lard	2.40	—	2.400	—	—	—
C.l.o.	0.15	—	0.150	—	—	—
Yeast	0.35	0.183	0.006	0.129	—	0.0310
Bone ash	0.40	—	—	—	—	0.4000
Salt Mixt.	0.20	—	—	—	—	0.2000
Tomato	2.00	0.024	0.004	0.080	1.880	0.0120
Lettuce	2.00	0.024	0.006	0.058	1.894	0.0180
Total	40.48	4.764	3.876	6.046	24.720	0.9887
—water	24.72					
Solid weight	15.76					
% by weight	—	30.2	24.6	38.4	—	6.3
Calories per kilo. total of 78	—	19	35	24	—	—

Nutritive ratio 1:3.1 .762 grams nitrogen per kilo.

In the case of the egg diet, the egg used was a pure, dried whole hen's egg.[1] One pound of dried egg is equivalent to approximately three and one-half dozen shell eggs. Due to the high fat content of the egg, this diet contained no lard, the fat being nearly entirely supplied by the egg. In preparing the egg for feeding, the necessary weighed amount of dried flakes was covered with water and allowed to stand in the ice box over night. It was then in a state similar to that of freshly beaten eggs.

The meat for the other diets was obtained fresh every two or three days. The visible fat and connective tissue were removed, and the meat ground and fed in this manner in connection with the other constituents of the diet.

The sucrose and corn starch supplied the necessary carbohydrates of the diets, while lard was used to supply the additional fat needed.

Patch's cod liver oil was used to insure a good supply of vitamins A and D, and incidentally make up the fat quota; Fleischmann's dried baker's yeast to insure the presence of vitamins B and G; tomato juice pressed from canned whole tomatoes to supply principally vitamin C; and chopped fresh green lettuce as a source of vitamin E.

A good grade of bone ash supplied roughage (to insure firm fecal matter) and helped make up the ash percentage.

The salt mixture used was that suggested by Cowgill (1923) as shown in Table VIII.

TABLE VIII

Salt	Grams	Per cent
NaCl	.1050	38.0
Mg citrate	.0905	32.5
KH_2PO_4	.0336	12.2
$CaHPO_4 \cdot 2H_2O$	.0215	7.8
KCl	.0192	7.0
Fe citrate	.0050	1.8
KI	.0013	0.5

The increasing caloric need, due to the growing fetuses, was met by increasing the entire diet 20 per cent at the beginning of the ninth week of pregnancy. From then on, to meet the demands of lactation, the diet was stepped up as follows:

At parturition .... 25% increase over maintenance
Start of 2nd week of lactation .... 50% increase over maintenance
Start of 3rd week of lactation .... 75% increase over maintenance
Start of 4th week of lactation .... 100% increase over maintenance

By studying the nitrogen balances and the mother's weight changes over various periods it seemed, if we can accept a positive nitrogen balance and a maintained weight as criteria of sufficient food requirement, that this change was sufficient to meet the increasing metabolic demands of the mother.

RESULTS

The results appear in the following order: Breeding, nitrogen balances, urinary analyses, blood analyses, milk analyses and growth changes.

1. Breeding and Number of Pups

Experiment 1, Dog 1, Diet 1 (egg). This dog was bred to male No. 1,

[1] Veritas, sold by Jaburg Bros. Inc., New York City.

Dec. 16, 1927. She delivered four pups, two males weighing 310 grams and 290 grams, and two females weighing 205 grams and 266 grams, respectively, on Feb. 15, 1928. The first three of these pups were used in the experiment.

Experiment 2, Dog 1, Diet 2 (liver). This dog was bred again to male No. 1, July 2, 1928. She delivered five pups, three males weighing 275, 260 and 290 grams, and two females weighing 275 and 290 grams, respectively, on September 4, 1928. The first male and the first female were discarded.

Experiment 3, Dog 1, Diet 1 (egg). This same dog was bred again to male No. 1, March 4, 1929. She delivered five pups, two males weighing 250 and 290 grams and three females weighing 255, 271 and 227 grams respectively, on May 3, 1929. The first male and the last female were discarded.

It was thought that if we could repeat the results of experiment 1, after dog 1 had been through experiment 2 (liver), we would be able to see if the diet was the main reason for the changed milk production, or whether the changed milk production was due solely to the betterment in lactation following a succeeding pregnancy. It is quite important to determine whether we are here dealing with changes in lactation due to a different age and parity, or to nutritional changes. We were unable to obtain much experimental support for the supposition that diet is the sole cause of changed milk production in this particular experiment, due to the development of infection in the mother and pups. Several of the pups developed convulsions. Owing to the infection we were unable to obtain any milk during the fifth week of lactation. We do not believe these pathological changes to be due to the diet.

Experiment 4, Dog 1, Diet 2a (liver). Dog 1 was bred again to male No. 1, April 2, 1930. She delivered five pups, two males weighing 210 and 304 grams and three females weighing 300, 274 and 264 grams, respectively, on May 31, 1930. The first male and the first female were discarded.

Since diet 2 (liver) contained a slightly greater amount of nitrogen per kilo. it was thought that this might account, in part, for the better results obtained in experiment 2 as compared with experiment 1, so in this second experiment on liver the diet was altered to conform more nearly to diet 1.

Experiment 5, Dog 2, Diet 3 (round). This dog was bred to male No. 3 about July 29, 1928, having been purchased in a pregnant state. She delivered seven pups, four females weighing 193, 213, 213 and 195 grams and three males weighing 206, 219 and 227 grams respectively, on Sept. 21, 1928. The first three females and the first male were discarded.

This dog was not a thoroughbred Boston bull terrier, but was probably

a mixture of French bull and Boston bull. She was bred to a large American bull terrier. For this reason we may not be justified in drawing an exact comparison between this experiment and the experiments on dog 1.

Experiment 6, Dog 9, Diet 4 (kidney). This dog was bred to male No. 4, Dec. 28, 1928. She delivered by caesarian section seven pups, one male weighing 200 grams and six females weighing 220, 235, 230, 205, 265 and 210 grams respectively, on Feb. 26, 1929. The mother was found dead the next day. The pups were given to a lactating American bull dog (dog 11) which had just weaned a litter of pups. She was fed on diet 4 until March 17, 1929 and samples of milk were taken for analysis. On March 17 she was changed to diet 3. Milk was taken for analysis on March 26. Her milk glands dried up on March 30. The analyses of the milk while under the influence of diet 3 are probably of no value since the mother was beginning to dry up.

There were four incomplete experiments conducted on diet 4 (kidney).

TABLE IX

Nitrogen balances. Experiment 1, Dog 1, Diet 1.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 2/4–2/12/28 inc. Total of 9 days. 20% inc. on 6th day.	gm. 61.98	39.23	7.30	—	+15.45	24.93
3rd week of lact. 2/29–3/6/28 inc. Total of 7 days.	77.48	36.06	9.11	Pups	+32.31	41.70
5th week of lact. 3/14–3/20/28 inc. Total of 7 days. Rejected 21.55 grams nitrogen of food	67.00	30.22	6.16	20.22	+10.40	15.52
Sexual rest. 5/11–5/17/28 inc. Total of 7 days. Rejected 12.65 grams nitrogen of food	31.62	28.29	3.06	—	+ 0.27	0.85

Note: Even though this dog did not eat for approximately two days during the fifth week of lactation and two days during the sexual rest period, she maintained a positive nitrogen balance.

In every case abnormal conditions developed at parturition causing the termination of the experiment.

2. *Nitrogen Balances*

The nitrogen balances were done primarily to see whether the increased food intake was meeting the metabolic demands in the various periods. This increase seemed to be sufficient because the experiments showed a positive nitrogen balance for all periods. The ingestion of the pups' excreta during the lactation period did not seem greatly to affect the nitrogen output. The balances in the third week of lactation are not "exact" because the mothers were secreting milk—a means of nitrogen elimination that was not deducted from the intake. However, in the fifth week of lactation when the output in the milk was considered, the animals were still in positive balance. There are greater percentage retentions of nitrogen on the liver diets as compared with the egg diet, thus indicating a better absorption and utilization of protein on the liver diets. This may be associated with the better milk production. Tables IX, X, XI and XII show the nitrogen balances.

TABLE X

Nitrogen balances. Experiment 2, Dog. 1, Diet 2.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 8/21–8/28/28 inc. Total of 8 days. Rejected 9.50 grams nitrogen of food	59.94	37.63	3.98	—	+18.33	30.58
3rd week of lact. 9/21–9/26/28 inc. Total of 6 days. Rejected 1.51 grams nitrogen of food	74.43	31.55	4.07	Pups	+38.81	52.14
5th week of lact. 10/4–10/10/28 inc. Total of 7 days. Rejected 20.21 grams nitrogen of food	81.06	36.20	4.21	26.96	+13.69	16.89
Sexual rest. 12/1–12/6/28 inc. Total of 6 days.	43.40	35.52	2.93	—	+ 4.95	11.40

Note: Even though this dog rejected approximately one day's food in each of the first three periods, she retained a positive nitrogen balance.

TABLE XI

Nitrogen balances. Experiment 4, Dog 1, Diet 2a.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 5/23–5/28/30 inc. Total of 6 days. Rejected 7.60 grams nitrogen of food	38.96	26.04	2.43	—	+10.49	26.93
3rd week of lact. 6/16–6/21/30 inc. Total of 6 days.	66.46	26.77	4.35	Pups	+35.34	53.17
5th week of lact. 6/30–7/5/30 inc. Total of 6 days.	75.96	30.59	4.78	28.45	+12.14	15.98

TABLE XII

Nitrogen balances. Experiment 5, Dog 2, Diet 3.

Period	Food	Urine	Feces	Milk	Balance	% Retention
9th week of preg. 9/18–9/21/28 inc. Total of 4 days.	31.08	11.72	0.92	—	+18.44	—
3rd week of lact. 10/8–10/13/28 inc. Total of 6 days.	67.99	26.14	3.96	Pups	+37.89	55.73
5th week of lact. 10/21–10/27/28 inc. Total of 7 days.	90.65	47.66	4.70	13.42	+24.87	27.43
Sexual rest. 12/15–12/21/28 inc. Total of 7 days.	45.32	31.68	2.39	—	+11.25	24.82

Note: Because the date of parturition could not be definitely estimated, the nitrogen studies for the pregnant period in this dog are not absolutely valid. The mother delivered before the metabolism period could be closed by the regular method; thus some feces and urine may have been omitted.

The results of this experiment probably are not to be compared with those of experiments 1, 2, 3, and 4, as has been stated, since this experi-

ment employed a different dog of different breeding. Table XII is shown to demonstrate that the adequacy of the diet is not peculiar to the individual dog for here with a dog of different breed and breeding we see a positive nitrogen balance throughout. The data indicate a high percentage retention of nitrogen and degree of utilization of round steak in this dog.

3. *Urinary Analyses*

In the study of the urinary chemistry we must bear in mind the fact that the intake was not the same for all four periods, though the percentage composition of the diet remained the same.

The slight increase in the nitrogen constituents of the urine, noted in some of the experiments during lactation, is probably due to the increased total nitrogen intake and the mothers ingesting the pups' excreta. The increased nitrogen intake probably is reflected also in the figures for sulfur and phosphorus. The ammonia and amino acid nitrogen figures are slightly higher during lactation. We know of no significance to attach to these results other than that they may be accounted for by the mothers ingesting the pups' excreta in the third week of lactation.

4. *Blood Analyses*

The blood analyses were done primarily to see whether there would be any significant changes in the blood chemistry due to the influence of the different dietary proteins during the lactation period. The blood sugar levels fall pretty well within the normal limits in all stages of the experiments. However the blood sugar in all cases shows a rise in the third week of lactation varying from 2 to 23 mgms. This change probably is not regular or large enough to be of any significance. There seem to be no significant changes in the non-protein nitrogen content of the blood. There is a slight rise during lactation in nearly every case. This slight rise is apparent also in the ammonia nitrogen during lactation as compared with pregnancy. As far as the corpuscular and plasma volumes are concerned, there seems to be an increased hydration of the blood during lactation. Experiment 3 shows that during the fifth week of lactation, this hydration is not apparent. There was an infection of the mammary gland causing an early drying up of the milk supply. This would lead us to believe that there is some relation between the hydration of the blood and milk production. There is a partial indication that blood lipids are higher in pregnancy and lactation in preparation for milk fat. The rise that is apparent in some cases may be only a reflection of the increased consumption of food fat during the later part of pregnancy and during lactation. If there is a

TABLE XIII
URINARY ANALYSES (TOTAL GRAMS FOR 7 DAYS)

Experiment	Dog	Diet	Analysis for	9th week Pregnancy	3rd week Lactation	5th week Lactation	Sexual Rest
1	1	1 (egg)	Total N	30.51	36.06	30.21	28.29
			NH_3 N	1.38	3.54	3.03	1.38
			Amino N	2.48	4.84	3.25	2.42
			Total S	6.26	9.30	7.08	6.91
			Total P	3.83	5.43	4.05	2.80
2	1	2 (liver)	Total N	32.93	36.80	36.19	41.44
			NH_3 N	2.14	2.22	2.33	0.81
			Amino N	2.45	3.13	3.22	2.38
			Total S	5.93	6.19	6.78	7.19
			Total P	4.00	6.04	5.46	4.24
3	1	1 (egg)	Total N	24.20	31.20	21.93	24.85
			NH_3 N	0.90	1.97	1.63	1.20
			Amino N	2.38	2.89	2.13	1.76
			Total S	6.40	8.16	7.46	
			Total P	2.80	3.29	2.48	2.38
4	1	2a (liver)	Total N	30.38	31.24	35.67	
			NH_3 N	1.80	2.39	2.39	
			Amino N	2.04	2.86	2.83	
			Total S	4.51	5.66	6.26	
			Total P	3.50	4.57	5.60	
5	2	3 (round)	Total N		30.49	47.66	31.68
			NH_3 N		0.58	1.16	1.02
			Amino N		0.77	1.80	0.95
			Total S		4.96	8.05	4.73
			Total P		1.47	2.43	1.75
6	9	4 (kidney)	Total N	31.17			
			NH_3 N	0.87			
			Amino N	1.36			
			Total S	5.61			
			Total P	2.73			

quantitative relationship between the quantity of blood lipids and the percentage amount of fat in the milk, it is not at all borne out in these experiments because the blood lipid values are all higher on the egg diet than on either the round or liver diets. Very slight changes in the quantity of fat in the diet have no influence upon the fat content of the milk. The diet containing the most fat and showing the highest blood lipid value produced milk with the lowest fat content.

TABLE XIV
BLOOD ANALYSES (MG. PER CENT)

Experiment	Dog	Diet	Analysis for	9th week Pregnancy	3rd week Lactation	5th week Lactation	Sexual Rest
1	1	1 (egg)	Sugar	88.89	111.11	100.00	88.90
			N.P.N.	30.00	26.08	30.77	23.10
			Amino N	7.00	5.55	8.09	8.90
			Corpuscles %	45.00	35.00	36.00	44.00
			Plasma %	55.00	65.00	64.00	56.00
			Tot. Lipids	730.57	975.50	992.40	631.00
2	1	2 (liver)	Sugar	108.00	111.00	87.00	90.00
			N.P.N.	24.00	33.30	30.00	35.70
			Amino N	7.77	11.00	11.60	11.60
			Corpuscles %	38.70	33.33	37.00	40.00
			Plasma %	61.30	66.66	63.00	60.00
			Tot. Lipids	594.59	468.74	551.52	523.64
3	1	1 (egg)	Sugar	91.00	93.00	87.00	90.00
			N.P.N.	27.27	37.50	36.36	33.30
			Amino N	9.30	11.60	13.30	8.90
			Corpuscles %	35.00	34.00	46.00	45.00
			Plasma %	65.00	66.00	54.00	55.00
			Tot. Lipids	729.00	545.00	755.00	
4	1	2a (liver)	Sugar	88.80	111.10	104.00	
			N.P.N.	22.41	25.00	24.00	
			Amino N	7.49	9.79	10.00	
			Corpuscles %	35.30	34.00	37.50	
			Plasma %	64.70	66.00	62.50	
			Tot. Lipids	663.80	646.90	775.31	
5	2	3 (round)	Sugar		96.30	93.80	93.80
			N.P.N.		24.00	27.20	38.70
			Amino N		8.75	8.40	11.60
			Corpuscles %		33.00	38.00	48.00
			Plasma %		67.00	62.00	52.00
			Tot. Lipids		405.40	461.99	444.25
6	9	4 (kidney)	Sugar	102.00			
			N.P.N.	35.20			
			Amino N	8.75			
			Corpuscles %	28.00			
			Plasma %	72.00			
			Tot. Lipids	622.00			

5. *Milk Analyses*

Probably the most striking feature is the quantitative collection. On the liver diets (2 and 2a) 1934 cc. and 2159 cc. respectively were collected over a week's period as compared with 1361 cc. collected on the egg diet and 865 cc. on the round diet.

Since we think of the percentage fat composition of the milk along with the quantity secreted, we find a higher fat percentage (13.5% and 13.25%) in the milk produced by the liver diets (2 and 2a) than that (12.25%) produced by either egg (diet 1) or round (12.5%) (diet 3). The other changes in the composition of the milk may or may not be significant. It is interest-

TABLE XV
MILK ANALYSES (GRAMS PER CENT)

Experiment	Dog	Diet	Analysis for	3rd week Lactation	5th week Lactation
1	1	1 (egg)	Sp. Gr.	1.033	1.030
			Fat	12.75	12.25
			Total solids	23.58	22.65
			Ash	1.16	1.24
			Proteins	7.74	9.46
			Casein		5.62
			Albumin		2.32
			Lactose	3.79	3.20
			Vol. in cc. for 7 days		1361.0
2	1	2 (liver)	Sp. Gr.	1.039	1.037
			Fat	13.25	13.50
			Total solids	22.90	22.50
			Ash	1.04	1.11
			Proteins	7.10	8.88
			Casein		5.42
			Albumin		2.03
			Lactose	3.10	3.14
			Vol. in cc. for 7 days		1934.0
3	1	1 (egg)	Sp. Gr.	1.029	
			Fat	11.00	
			Total solids	21.52	
			Ash	0.96	
			Proteins	6.27	
			Casein	4.08	
			Albumin	1.46	
			Lactose	2.92	

TABLE XV (*Continued*)

Experiment	Dog	Diet	Analysis for	3rd week Lactation	5th week Lactation
4	1	2a (liver)	Sp. Gr.		1.032
			Fat		13.25
			Total solids		23.50
			Ash		1.07
			Proteins		8.74
			Casein		5.20
			Albumin		1.79
			Lactose		3.21
			Vol. in cc. for 7 days		2159.0
5	2	3 (round)	Sp. Gr.	1.035	1.039
			Fat	10.75	12.50
			Total solids	29.20	25.40
			Ash	1.61	1.33
			Proteins	6.34	9.70
			Casein		7.03
			Albumin		2.07
			Lactose	3.39	2.79
			Vol. in cc. for 7 days		865.0
6	11	4 (kidney)		After feeding kidney 19 days	
			Sp. Gr.	1.039	
			Fat	10.25	
			Total solids	21.53	
			Ash	1.22	
			Proteins	7.66	
			Casein	4.92	
			Albumin	1.78	
			Lactose	3.60	

ing to note, however, that the protein content is higher in the fifth than in the third week of lactation, while the total solids are slightly lower in each case. The protein content and the ash content of "egg" milk are slightly higher than those of "liver."

In experiment 6 we see that the foster mother (dog 11), when nursing the five pups of dog 9 and being fed kidney (diet 4) produced milk showing a fat content of 10.25 per cent. This experiment, of course, is not at all comparable with the others but was done in order to gain some light on the composition of "kidney" milk. It was believed that kidney might be an excellent milk stimulant. It evidently provided plenty of milk for

this mother to raise five pups after having raised a large litter of her own. The disappointing fact was that the milk did not contain as high a fat content as was expected. Since this dog was not a Boston bull, but an American bull (much larger) it is likely that these dogs do not produce a milk with so high a fat content, and that this 10.25 per cent may be in the high range of its capacity. The work quoted from Voit (1869) on the milk analysis of a dog weighing 34 kilos. showed a fat percentage of only 7.39 per cent to 10.32 per cent. It is possible that the larger species of dogs produce milk of a lower fat content than dogs of a much smaller breed, such as Boston bull dogs.

When dog 11 was changed to round diet (diet 3) she rapidly dried up. This may have been coincidental or it may have been due to the inefficiency of the round diet.

6. Growth Changes

We note, from the plotted growth curves of the pups (Chart 1), that the liver diet again excels. The pups in experiments 2 and 4 (liver) showed much better growth than those in the other experiments, (egg, round and kidney).

Experiment 4 duplicates experiment 2 and experiment 3 duplicates experiment 1 as far as the growth curve could be studied (infection in the latter part of experiment 3). This seems to prove that the changes in lactation were solely due to dietary changes and not the age and parity of the mother.

Chart 1 also shows that round is comparable with egg as a stimulant. Kidney is probably the least effective. These findings may not be exactly true since experiments 5 and 6 were carried out on different dogs and since the mother in experiment 6 was only a foster mother and nursed five pups instead of three.

Table XVI gives a comparison of the *m* values. We see that the difference between the average *m* for the liver diets and that for the egg diets is quite significant and that the liver is 1.34 times as good as egg, or in terms of per cent one can say that egg is only 74.57 per cent as good as liver for milk production, as judged by the growth of the pups.

If we regard the experiments done on different dogs (experiments 5 and 6) and if we are at all justified in comparing them with the first four experiments, we see that "round" ranks with "egg" and "kidney" ranks much lower. The *m* value for "kidney" undoubtedly would have been different had there been three instead of five pups.

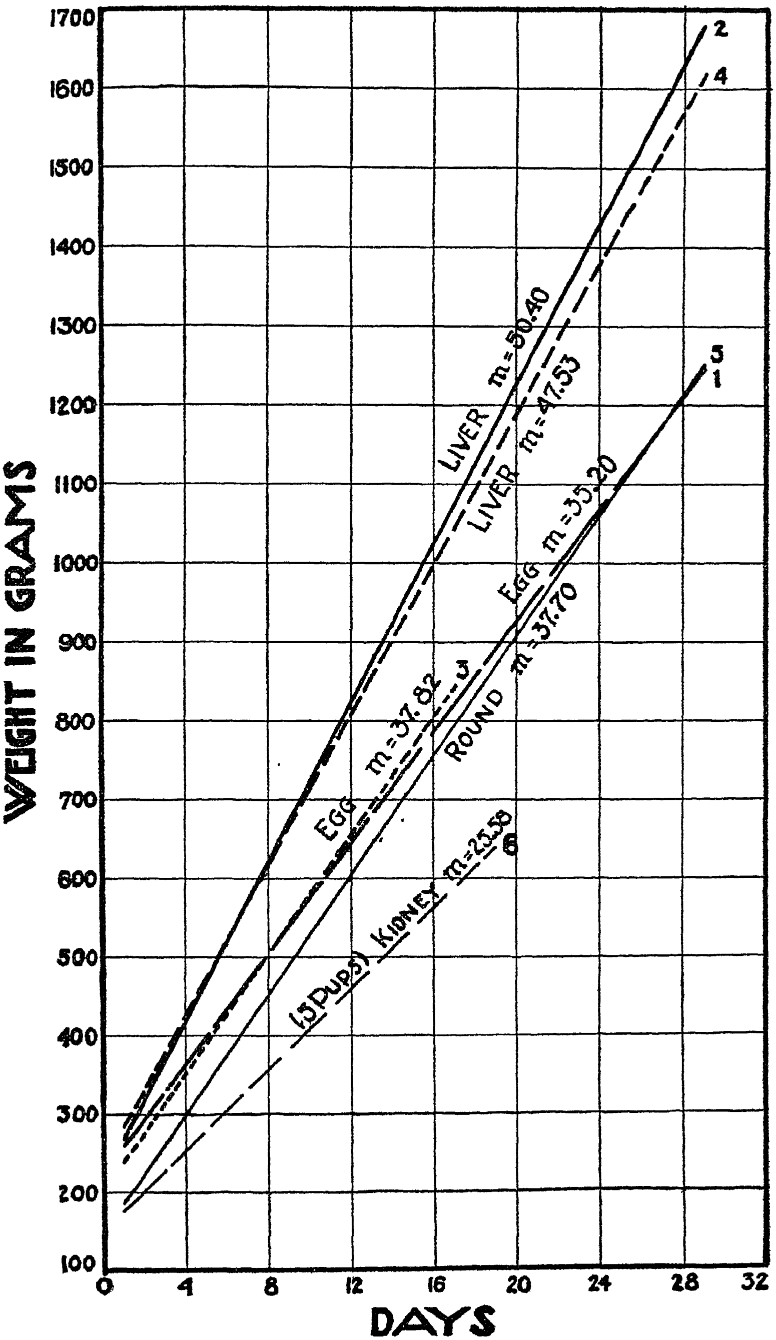

CHART 1.—The growth curves were plotted according to the method of least squares using the formula $y=mx+b$ where: $m=\frac{n\Sigma xy-\Sigma x\Sigma y}{\dots}$; $b=1/n(\Sigma y-m\Sigma x)$; x=days old;

General Discussion

Undoubtedly breeding is reflected in the quality and quantity of the milk production. It also is linked with the growth changes of the offspring. Knowing these facts and the fact that there are so many variables in connection with the experimental study of lactation, we are truly justified in drawing definite conclusions only from experiments 1, 2, 3 and 4 where the same female dog was bred to the same sire and kept under identical

Table XVI
Growth Analysis

Expt.	Diet	m*	Aver.	Ratio of m Liver:Egg
2	Liver (2)	50.40	48.96	
4	" (2a)	47.53		
3	Egg (1)	37.82	36.51	1.341:1
1	" (1)	35.20		
5	Round (3)	37.70		
6	Kidney (4)	25.58	(5 pups)	

* The value m designates the slope of the curve or the rate of change of y with respect to x, calculated from the formula $y=mx+b$.

conditions in all four experiments with the exception of the source of protein in the diet. Using this set of conditions as a basis, we can say that liver is a better food protein than egg for lactation in the dog. This statement is substantiated by the following experimental findings.

There are greater percentage retentions of nitrogen on the liver diets indicating a better absorption and utilization of the liver protein.

The liver diet is responsible for the production of more and better milk. The amount of milk produced is not correlated with the number of pups in utero or the number of pups nursed, but rather with the protein changes in the diet.

The growth rate of the pups when the mother was fed liver is 1.34 times that of the pups when the mother was fed egg.

The question as to why liver is better than egg for the production of milk can not be answered from the data at hand. However, one may speculate as to the reason. It may be that liver is more appealing to the dog, since the dog is more accustomed to eating meats. There seems to be a better utilization of liver. This probably reflects itself in the milk production. It also may be that liver is a more generalized and more complete protein than egg for dogs. The amino acid distribution in milk may be more nearly similar to that found in liver than to that found in egg. Since the

quantitative figures for the entire amino acid distribution in these proteins are not available at present, we are unable to say whether or not this factor is responsible. Still another hypothesis is that liver may contain some hormone or lactation-promoting vitamin in greater abundance than does egg.

Special Remarks on the Kidney Diet

Of all of the experiments started on kidney (diet 4) none was successful (other than the freak experiment 6). Whether these failures were brought about by the toxicity of the kidney in pregnancy or by mere coincidence is a question. The kidney diet did not seem at all toxic to the lactating dog 11. Concerning the toxicity of the kidney let us consider the manner of preparation of the diet. Fresh beef kidneys were obtained, fat and connective tissue removed, and the meat washed with water to insure the removal of any adherent urine. The kidney was then ground and mixed with the other constituents of the diet just before feeding. It is rather unlikely that the kidney had any toxic effect upon the organism. McCollum, Simmonds and Parsons (1921) in studying the nutritive properties of certain animal tissues state that there is no distinct evidence of toxicity in either muscle, kidney or liver tissue when fed at planes of intake sufficiently high to introduce from 35 to 70 per cent of protein in the diet of rats. These same workers and also Hoagland and Snider (1926) have shown that kidney, liver and muscle have approximately identical nutritive values for rats. These results may or may not be applicable to dogs. Another supposition is that the kidney stimulated the intra-uterine growth thus causing the pups to be much larger than they would have been normally and so preventing natural delivery at parturition. In many cases there were deformed fetuses and in some cases hydrocephalic pups. In all except one case there were quite enlarged mammae in the last part of pregnancy, indicating the promise of an abundant milk supply. In all cases the mother was unable to deliver her young normally. This may have been coincidental since we know that they were all primiparas and that the per cent of Boston bulls that are unable to deliver normally is high. Assuredly we are not altogether justified in condemning kidney diet.

Summary and Conclusions

Balanced diets, containing all of the known vitamins and other requirements, were fed to Boston bull females of the same breed and breeding. The diets were all based upon the following scheme: 0.7, to 0.8 gram of nitrogen per kilo., 75 to 80 calories per kilo., 30 per cent protein, 25 per cent fat, 40 per cent carbohydrate and 6 per cent ash. The only variable

in the diets was the source of protein. Beef liver, round, kidney and dried hen's egg were the sources of protein.

The experimental diet was fed from the time of conception through the fifth week of lactation, the diet being increased to meet the demands of the growing pups.

The litters were all limited to three pups and the mothers given identical care.

The milk was collected by manual manipulation during the fifth week of lactation when the pups were not with the mother.

One dog was bred to the same sire and kept under identical conditions for four pregnancy-lactation periods. Egg was the source of protein in the first and third periods while liver was used in the second and fourth.

She showed a better nitrogen retention on liver both times. When fed liver she produced in the fifth week of lactation 1934 cc. of milk, containing 13.5 per cent fat in one experiment and 2156 cc. of milk, containing 13.25 per cent fat in the other. When fed egg she produced in the corresponding week, only 1361 cc. of milk containing 12.25 per cent fat. The pups of the liver experiments grew 1.34 times as fast as those of the egg experiments.

Another dog of a different breed and breeding, when fed round steak as the source of protein, produced in the fifth week of lactation 865 cc of milk containing 12.5 per cent fat. The growth of the pups was practically the same as on the egg experiment.

Owing to some unknown cause, no experiments were completed on kidney diet. This diet did not seem toxic and produced plenty of milk when fed to a lactating dog with five pups. It may have some effect on the intra-uterine life of the pups, since none of the mothers fed on this diet during pregnancy delivered normally.

The chemical studies may be of some significance in the study of the physiology of lactation.

The work shows the degree of betterment in lactation that can be brought about by changing the source of protein in the diet. Liver is a much better source of protein for lactation than either egg or round steak.

Our conclusion that liver is the best source of protein (of those tried) for milk production in dogs, is borne out by the quantity of milk produced, the fat content of the milk, the nitrogen retention and the slope of the growth curve of the pups.

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THE VALUE OF THE OYSTER IN NUTRITIONAL ANEMIA

By

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SINCE the anouncement of Hart and co-workers (1) in 1928 that copper is effective in supplementing iron in the cure of anemia produced by feeding milk to the rat, considerable interest has been manifested in the possible supplementary rôle of various other inorganic elements. Although all other investigators (2 to 9, 43) in this field, except Drabkin and Waggoner (10) who question the specificity of copper, agree that copper supplements iron in the regeneration of blood in nutritional anemia, several workers (5, 7b) offer evidence tending to show that other elements besides copper are just as effective in their supplementary action and further (6, 7a) that a group of inorganic elements rather than any single element is concerned in blood regeneration. In these investigations, the rôle of manganese has received particular attention.

As a result of these studies, much confusion now exists in the literature as to what elements are concerned in hemoglobin regeneration in nutritional anemia. Thus, Waddell and co-workers (8) report that Cu is "unique" in supplementing Fe and that Zn, Cr, Ge, Ni, Co, Pb, Sb, Sn, Cd, Hg, As, and Mn, when fed either singly or as a mixture of these twelve elements, are ineffective. Lewis, Weichselbaum and McGhee (4) have found Mn and Co to be ineffective while Krauss (9) has recently confirmed Waddell in respect to Mn. Likewise, Underhill and co-workers (43) have recently demonstrated the ineffectiveness of Co, Ni, Zn, and Mn. On the other hand, Myers and Beard (5a, b) offer results showing that besides Cu, Mn as well as Ni, Ge, Co, As, Ti, Zn, Rb, V, Cr, Se, and Hg are effective. Goerner (2) found Mn to be potent, while Zn and Al are reported as inert. Again, Titus, Cave and Hughes (7a, b) have reported that Mn is just as effective as Cu, whereas, in accord with the results of Mitchell and Miller (6), they found a combination of Mn and Cu to be more potent than Cu alone in supplementing Fe.

This interest in the inorganic elements, particularly in Fe, Cu, and Mn,

led several laboratories to carry out analyses for these three elements on a large number of plant and animal foods. The Wisconsin workers (11 to 16) have been very active in this direction and have published results showing the Fe, Cu, and Mn content of a wide variety of both plant and animal foods. Richards (17) likewise gives values for the Mn content of various foods. In this laboratory, Remington and Shiver (18) determined the amounts of Fe, Cu, and Mn in a large number of samples of eighteen of the more commonly used vegetables grown in South Carolina. At the same time, the laboratory became interested in making similar determinations on various animal foods. In this connection attention was drawn to the oyster, which we found to contain rather large amounts of both Fe and Cu. The latter element was present in much greater concentration than was found in any plant or animal food thus far examined here. Further analysis revealed the presence of manganese also. It occurred to us, therefore, that since the oyster is such a good source of both Fe and Cu (elements admitted to be concerned in hemoglobin regeneration in nutritional anemia), it ought to exhibit marked potency as an hematopoietic agent.

A review of the literature revealed that the oyster, besides being a good source of vitamins A, B, C and D (19 to 23), contains in rather high concentration various inorganic elements, of which all except iodine have been studied in nutritional anemia. The amounts of iron (13), copper (15, 24 to 29), zinc (27, 29 to 32, 35), lead (34), arsenic (27, 33) and iodine (36, 37) contained in the oyster have been determined by several investigators. Besides these, other metallic elements are probably present.

The presence of these several elements again suggested that the oyster might be a potent anti-anemic food, and hence the present study was undertaken. Since in addition to Fe, Cu, and Mn, other elements—Zn, Pb and As—are present, we decided to compare the effectiveness of the oyster with a solution containing equivalent amounts of Fe, Cu, and Mn alone. By this procedure we reasoned that if elements other than Fe, Cu, and Mn play a rôle in blood regeneration, then the oyster would be much more effective than the solution of the three inorganic elements. In addition, an acid solution of oyster ash was fed for comparative purposes. It was expected that the results of this experimental plan would throw further light on the existing controversy as to the elements concerned in blood regeneration in nutritional anemia.

Experimental

The rats used in the experimental work were bred in the laboratory em-

ploying Sherman Diet 13 as modified by Russell (38). The composition of the breeding ration follows:

	Per Cent
Whole wheat (ground)	59.8
Whole milk powder*	29.9
Swift's meat scrap (55% protein)	9.1
Sodium chloride	1.2
	100.0

* Klim.

This ration was found to contain 125 mg. of Fe and 4.1 mg. of Cu per kg. Distilled water was given *ad libitum* to all rats. Shortly after birth, the number of rats in each litter was reduced to seven. At 21 days of age, the young rats in our colony averaged 36 grams in weight. The animals were allowed to remain with the mother until they reached an average weight of 55 to 60 grams, which they usually attained at 25 to 30 days of age. They were then separated from their mothers, grouped by litters in galvanized iron wire cages and fed exclusively on milk in order to render them anemic. At the same time, rats taken from these same litters were maintained on the stock ration throughout both the preliminary and experimental periods as controls.

Following the usual technic (39) used by other investigators, blood samples were taken from the tip of the tail. The blood, after being drawn into the blood pipette, was expelled immediately into a small test tube containing 5 cc. of one per cent HCl solution and then shaken. When all the blood samples had been taken in this manner, the test tubes were heated at 55 to 60°C for seven minutes and cooled to room temperature. This procedure, used by Mitchell and Miller (6), insured maximum color development of the acid hematin. Hemoglobin determinations were made by the Newcomer method, using a calibrated yellow glass disc in the colorimeter as a standard. Following the experience of Waddell and co-workers (39), we dispensed with the taking of red blood cell counts in our investigation.

A total of seventy-three rats was used in the experimental work. In order to establish the average hemoglobin value of our animals at the start of the preliminary milk feeding period, five litters of rats comprising thirty-four animals were used. The average hemoglobin value for these rats was found to be 11.79 gm. per 100 cc. of blood. This value lies between the average value of 10.83 obtained by the Wisconsin workers (39) for rats 3 to 4 weeks of age weighing 50 to 60 g. and the value of 12.62 obtained by Williamson and Ets (40) for animals 20 to 39 days old.

In rendering the rats anemic, whole milk powder[1] reconstituted to liquid

[1] Klim.

milk was employed. Reconstitution was effected by adding the required amount of warm distilled water to the milk powder contained in a Mason jar. The jar was then closed securely and vigorously shaken by hand for about ten minutes.

The whole milk powder was purchased in fifty pound tins, each lot being analyzed[2] for iron and copper before using it for feeding purposes. In all, five lots of milk powder were used throughout the experimental work. The iron and copper content of the different lots of dry milk varied slightly. Expressed in terms of liquid milk, the values varied from 1.0 to 1.4 mg. per liter for Fe, and from 0.20 to 0.27 mg. per liter for Cu. Assuming an average daily intake of 35 cc. of milk per rat, this amount of milk would afford the animal 0.035 to 0.050 mg. of Fe and 0.007 to 0.009 mg. of Cu. The distilled water used in reconstituting the milk introduced only negligible amounts of Fe and Cu. Drabkin and Waggoner (10) also used powdered whole milk[1] to produce anemia in their rats.

The time required to produce a marked anemia on milk varied from 6 to 8 weeks. At the end of this time, the average hemoglobin value for all the milk-fed animals had decreased to 5.68 gm. per 100 cc. of blood, the individual values ranging from 3.19 to 7.80 gm. per 100 cc. Simultaneously, seven litter-mate control animals that had been kept on the stock ration for the same length of time yielded an average hemoglobin value of 15.3 gm. Hemoglobin determinations were also carried out on a group of twenty-eight stock rats representing four litters from our breeding colony. These animals (84 to 89 days old) were approximately the same age as the anemic rats at the end of the preliminary milk feeding period. The hemoglobin values for this group on the stock ration averaged 14.84 gm. per 100 cc. of blood with a range in values of 14.03 to 16.81 gm. per 100 cc. Williamson and Ets (40) reported a hemoglobin value of 13.54 for normal rats 80 to 99 days of age. The blood of our anemic rats at the start of the curative period contained only 38 per cent of the normal content of hemoglobin.

At the start of the curative period, the rats were placed in individual galvanized iron wire cages. There were altogether 66 anemic animals, of which number 16 rats, representing various litters, were retained on milk *only* throughout the curative period to serve as negative controls. The remaining 50 anemic rats were divided into groups and fed the various test materials to be described, while the seven animals fed the stock ration during the preliminary period were maintained on this same diet throughout the experimental period to serve as normal controls.

[2] All analyses for the iron, copper or manganese content of materials employed were carried out by the methods used by the Wisconsin workers (12 to 14, 41).

The oysters used in the present investigation were obtained from various beds located along the coast of South Carolina. Shucked oysters from these localities were brought to the laboratory, dried in porcelain evaporating dishes first over a steam bath and finally in an electric oven. Table I below shows the iron, copper and manganese values for six samples of oysters. A

Table I

Showing the Iron, Copper and Manganese Content (Dry Basis) of Oysters Grown Along the Coast of South Carolina

Sample No.	Source	Iron (p.p.m.)	Copper (p.p.m.)	Manganese (p.p.m.)
423	Charleston County (Bull's Bay)	760	93	34
648	Charleston County (Bull's Bay)	633	52	15
649	Beaufort County (Daufuski Island)	929	67	26
652	Beaufort County (Daufuski Island)	1035	40	58
653	Beaufort County (Bluffton)	740	34	46
731	Georgetown County (Winyah Bay)	510	28	29
Range in values		510–1035	28–93	15–58
Average value		768	52	35

study of the values in this table reveals that there is much variation among the samples. Hiltner and Wichman (27) in 1919 analyzed oysters from eight different beds situated at or near the localities indicated in Table I. Using the iodometric method for the determination of copper, these investigators reported values ranging from 23 to 52 p.p.m. (fresh basis). For comparison with the results of our copper analyses, these values would be, expressed on a dry basis, approximately 161 to 364 p.p.m. When the same samples were examined for zinc, Hiltner and Wichman found amounts ranging from 182 to 502 p.p.m. (fresh basis).

For the feeding experiments, a composite sample (Sample 1) representing a mixture of three dried oyster samples was made so as to have sufficient material to last throughout the entire experimental period. On analysis, the mixed sample yielded the following values: Fe, 892 p.p.m.; Cu, 58 p.p.m. and Mn, 20 p.p.m. In choosing the various levels of dried oysters for daily feeding to the rats, 0.56 gm. was selected as the highest dosage since this amount furnished 0.5 mg. of Fe and 0.0327 mg. of Cu—amounts which have been shown by different workers to be fully adequate for blood regeneration in nutritional anemia. Another dried oyster sample (Sample 2) was also used in the feeding tests, this sample containing 760 p.p.m. of

Fe, 93 p.p.m. of Cu and 34 p.p.m. of Mn. Table II shows the various levels at which the two samples were fed, together with the daily amounts of Fe, Cu and Mn furnished by each dosage.

TABLE II
SHOWING THE AMOUNTS OF FE, CU, AND MN (DRY BASIS) CONTAINED IN THE DAILY OYSTER SUPPLEMENTS

Sample No.	Amount of dried oysters	Equivalent on a fresh basis	Iron	Copper	Manganese
	gram	gram	mg.	mg.	mg.
1	0.56	4.0	0.500	0.0327	0.0112
1	0.28	2.0	0.250	0.0164	0.0056
1	0.14	1.0	0.125	0.0082	0.0028
2	0.16	1.7	0.125	0.0149	0.0054

For comparative purposes in the feeding tests, three concentrations of an HCl solution of the oyster ash of Sample 1 were prepared in such a manner that 1 cc. of each solution corresponded respectively in Fe, Cu, and Mn content to the three levels of dried oysters (Sample 1). Since besides Fe, Cu, and Mn other workers (27, 29 to 35) have found rather large amounts of Zn, Pb and As in the oyster—elements concerning the rôle of which Waddell and co-workers (8) and Myers and Beard (5a, b) offer contradictory evidence—a comparison of dried oysters (Sample 1) and of the oyster ash solution was made with a prepared solution[3] containing only Fe, Cu, and Mn, in order to determine whether or not elements other than Fe, Cu, and Mn are concerned in hemoglobin formation.

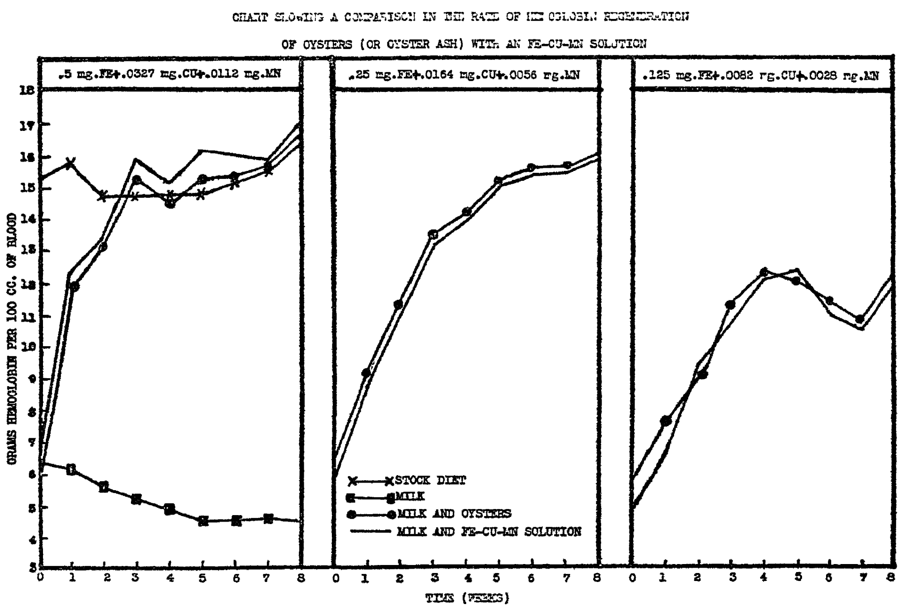
The mineral supplements were fed daily to the rats in the morning, at which time no milk was given until the supplement was entirely consumed. In the case of the 1 cc. liquid supplements, these adjuvants were mixed with about 3 to 5 cc. of milk before feeding. The rats consumed the supplements readily after which they were given milk *ad libitum* until the next morning. The experimental feeding period lasted eight weeks.

The results obtained in the feeding experiments are summarized in Ta-

[3] This solution was prepared in the following manner. Using C. P. iron wire, a $FeCl_3$ solution was made from which the Cu was precipitated by H_2S. $CuSO_4$ and $MnCl_2$ solutions were used to represent Cu and Mn. On analysis, these three solutions were found to contain negligible amounts of Fe, Cu, and Mn as contaminants. From these sources, solutions were prepared corresponding in Fe, Cu, and Mn content to the various levels of dried oyster and oyster ash solution. The required amount of the three elements was contained in 1 cc. of each solution.

ble III and are shown graphically in Chart 1.[4] From a study of Table III, it is to be observed that the average hemoglobin content of the blood of the sixteen negative control rats on milk alone decreased from 6.4 gm. per 100 cc. at the start of the experimental period to 4.5 gm. per 100 cc. at the end of this period at which time only nine rats survived. The average values for the group of seven normal control animals representing rats maintained on the stock ration throughout both the preliminary and experimental periods remained practically the same.

The data in the table also clearly indicate the marked effectiveness of the oyster in hemoglobin regeneration. The three levels of dried oyster Sample 1 (0.56 gm., 0.28 gm. and 0.14 gm.) all exhibited the capacity to



raise the hemoglobin content of the anemic rats' blood. Accepting for comparative purposes 14.8 gm. hemoglobin per 100 cc. as the average value for our normal animals, it is evident from a study of the values in the table that daily dosages of 0.56 gm. and 0.28 gm. of dried oysters permitted complete blood regeneration in 2 to 3 weeks and 4 to 5 weeks, respectively. On the other hand, a level of 0.14 gm., although it permitted a considerable degree of blood regeneration, nevertheless failed to bring the hemoglobin

[4] Since both dried oysters and oyster ash gave practically the same response in hemoglobin regeneration, their values are plotted as one group in the chart.

TABLE III

AVERAGE WEEKLY HEMOGLOBIN VALUES* AT TIME SUPPLEMENT WAS ADDED AND AT INTER-
FORM OF DRIED OYSTERS, AN ACID SOLUTION OF OYSTER ASH AND A SOLUTION CONTAINING ONLY

	Negative Controls on Milk	Normal Controls on Stock Diet	Sample 1: 0.500 mg. Fe, 0.0327 mg. Cu, 0.0112 mg. Mn — Furnished by: .56 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn
At time of addition†	6.4±.21 (16)‡	15.3±.32 (7)	5.0±.41 (4)	5.3±.28 (4)	6.0±.16 (4)
Weeks (after addition)					
1	6.2±.22 (16)	15.8±.32 (7)	11.8±.32 (4)	11.8±.58 (4)	12.2±.52 (4)
2	5.5±.20 (16)	14.7±.14 (7)	13.7±.06 (4)	12.7±.38 (4)	13.3±.38 (4)
3	5.2±.28 (15)	14.8±.22 (7)	15.0±.48 (4)	15.5±.14 (4)	15.9±.43 (4)
4	4.8±.24 (13)	14.7±.14 (7)	14.0±.21 (4)	15.1±.14 (4)	15.1±.35 (4)
5	4.5±.29 (12)	14.8±.34 (7)	15.5±.71 (4)	15.0±.24 (4)	16.3±.43 (4)
6	4.5±.27 (11)	15.2±.29 (7)	15.0±.50 (4)	15.4±.30 (4)	16.1±.38 (4)
7	4.6±.28 (10)	15.5±.11 (7)	16.2±.21 (4)	15.0±.48 (4)	15.7±.49 (4)
8	4.5±.28 (9)	16.4±.30 (7)	17.2±.69 (4)	16.3±.57 (4)	17.1±.51 (4)

* Expressed as grams of hemoglobin per 100 cc. of blood.

† After 6 to 8 weeks of preliminary feeding on reconstituted milk only.

‡ The figures in parenthesis refer to the number of rats used in obtaining the average hemoglobin value.

level up to the normal value. At the end of the eight weeks period, the average hemoglobin content of the blood of the rats fed this dosage reached only 12.0 gm. per 100 cc., representing approximately 80 per cent regeneration. A similar partial recovery was obtained with 0.16 gm. oyster Sample 2 which in comparison with 0.14 gm. of oyster Sample 1 furnished the same amount of Fe but about twice as much of Cu and Mn. At the conclusion

TABLE III (*continued*)

VALS THEREAFTER, OF ANIMALS RECEIVING VARIOUS AMOUNTS OF FE, CU, AND MN IN THE THE THREE ELEMENTS

Sample 1						Sample 2
0.250 mg. Fe 0.0164 mg. Cu 0.0056 mg. Mn			0.125 mg. Fe 0.0082 mg. Cu 0.0028 mg. Mn			.125 mg. Fe .0149 mg. Cu .0054 mg. Mn
Furnished by			Furnished by			Furnished by
.28 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn	.14 gm. Dried Oysters	HCl Soln. of Oyster Ash	Solution Containing Fe, Cu, and Mn	.16 gm. Dried Oysters
5.6±.23 (6)	6.3±.16 (6)	5.8±.20 (6)	6.3±.20 (5)	4.9±.24 (6)	4.8±.23 (6)	5.4±.27 (3)
9.7±.49 (6)	8.5±.28 (6)	8.9±.41 (6)	8.2±.38 (5)	7.4±.28 (6)	6.7±.25 (6)	8.5±.13 (3)
11.2±.49 (6)	10.9±.38 (6)	11.2±.57 (6)	8.9±.38 (5)	9.7±.30 (6)	9.4±.34 (6)	10.3±.35 (3)
13.5±.44 (6)	13.5±.18 (6)	13.2±.24 (6)	11.3±.30 (5)	11.4±.33 (6)	10.9±.26 (6)	11.1±.24 (3)
13.7±.18 (6)	14.4±.27 (6)	14.1±.35 (6)	12.6±.44 (5)	12.6±.38 (6)	12.1±.37 (6)	12.7±.26 (3)
15.0±.37 (6)	15.3±.33 (6)	15.2±.39 (6)	12.1±.48 (5)	12.1±.48 (6)	12.4±.57 (6)	11.7±.11 (3)
15.7±.48 (6)	15.5±.68 (6)	15.4±.55 (6)	11.5±.50 (5)	11.4±.39 (6)	11.0±.66 (6)	11.3±.35 (3)
15.7±.42 (6)	15.5±.18 (6)	15.5±.25 (6)	10.8±.50 (5)	10.7±.47 (6)	10.5±.65 (5)§	10.0±.47 (3)
16.5±.22 (6)	15.4±.07 (6)	16.0±.41 (6)	12.0±.70 (5)	12.5±.84 (5)§	12.1±.62 (5)	10.8±.32 (3)

§ One rat in this group died.

of the eight weeks' supplementary feeding period, 0.125 mg. of Fe (as $FeCl_3$ solution) was added to 0.16 gm. oyster Sample 2 thereby increasing the daily intake of Fe to 0.250 mg. This Fe augmentation permitted hemoglobin regeneration to the normal level in three weeks. This finding indicates that a daily dosage of 0.125 mg. of Fe as furnished by 0.16 gm. of dried oyster Sample 2 is insufficient for complete blood regeneration in the rat, despite the rather large amount of Cu—0.0149 mg.—which accompanied it. Further proof of this finding is indicated by the fact that 0.28

gm. of oyster Sample 1 which furnished approximately the same amount of Cu and Mn, but twice as much Fe as 0.16 gm. of oyster Sample 2, cured anemia in 4 to 5 weeks.

From the above results, it is evident that the minimum effective level of dried oyster Sample 1 lies between 0.14 gm. and 0.28 gm. or, on an undried basis, between 1.0 gm. and 2.0 gm. Since the amount of Fe furnished daily by the milk was approximately 0.035 to 0.050 mg. the results indicate that the minimum daily Fe requirement of the rat lies between 0.17 mg. and 0.30 mg. when supplemented with the amounts of Cu and Mn used in these experiments.

A further study of the data given in Table III shows that the dried oysters (Sample 1), the acid solution of oyster ash (Sample 1) and the prepared solution containing *only* Fe, Cu and Mn all brought about hemoglobin regeneration at approximately the same rate[5] when compared at the three different levels. This result indicates, first, that the inorganic elements present in the oyster are responsible for its hemoglobin regenerating capacity and second, that the antianemic potency of the oyster can be accounted for on the basis of its content of the three elements, Fe, Cu, and Mn.

Discussion

In the light of the controversy concerning the indispensability of elements other than Fe and Cu in blood formation, the above experimental results offer some pertinent evidence. Mitchell and Miller (6), in a study comparing a spinach extract fed at a level containing 0.5 mg. Fe, 0.014 mg. Cu and 0.012 mg. Mn with two prepared solutions containing in the one, 0.5 mg. Fe and 0.05 mg. Cu, and in the other, 0.5 mg. Fe, 0.05 mg. Cu and 0.05 mg. Mn, found the extract to be more potent in blood regeneration than the Fe-Cu or the Fe-Cu-Mn complex of pure salts. They also reported that the Fe-Cu-Mn complex was more effective than the Fe-Cu combination, indicating that Mn plays a rôle in hematopoiesis. When these same investigators subjected the spinach extract to qualitative analysis and found the following elements present—Cu, Sb, Sn, Fe, Al, Zn, Mn, Sr. Ca, Mg, Na, K and P—they concluded that rather than Cu alone there is a group of elements that is active in supplementing Fe in hemoglobin building. Myers and Beard (5a, b) also offer evidence indicating that several elements besides Cu and Mn are concerned in blood formation. On the other hand, the results of our investigation would appear to be more in

[5] Calculations of the significance ratios revealed that the differences between the mean values are not significant.

line with those of Waddell and co-workers (8) who in tests on several elements reported that Cu is "unique" in supplementing Fe. This contention appears evident since in the present experiments the prepared solution containing only Fe, Cu, and Mn allowed blood regeneration at approximately the same rate as the dried oysters and the oyster ash solution, despite the presence of Pb, Zn, and As in large amounts in the oyster. For this reason, the results obtained in this investigation would seem to warrant the conclusion that elements in the oyster other than Fe, Cu, and Mn are not concerned in blood formation.

The question whether or not Mn is necessary and aids Cu in supplementing Fe cannot be answered from the results obtained in the present experiments, but will be considered in another investigation now under way. Krauss (9) Underhill and co-workers (43) and Waddell and co-workers (8) give evidence showing that Mn is ineffective in supplementing Fe, whereas Myers and Beard (5a) and Titus, Cave and Hughes (7a) are of the opinion that Mn plays a rôle in the cure of nutritional anemia.

Sherman (42) estimates the Fe requirement of man as 15 mg. per day. On this basis, 120 g. (4.2 oz.) of oysters (undried) would furnish this daily requirement.

Summary

The finding of large amounts of Cu in the oyster prompted a study of the influence of the oyster in hemoglobin regeneration in rats rendered anemic on milk.

When fed daily for a period of eight weeks, 0.56 gm. dried oysters (4.0 gm. fresh basis) allowed regeneration to the normal hemoglobin level in 2 to 3 weeks, whereas 0.28 gm. (2.0 gm. fresh basis) brought a return to normal in 4 to 5 weeks. Fourteen hundredths of a gram (1.0 gm. fresh basis) permitted only about 80 per cent blood regeneration at the end of eight weeks. It is estimated that the minimum daily Fe requirement of the rat lies between 0.17 mg. and 0.30 mg.

A comparison of 1.–dried oysters, 2.–an acid solution of oyster ash and 3.–a solution containing Fe, Cu, and Mn, was made at various levels. All three supplements permitted blood regeneration at approximately the same rate, indicating, first, that inorganic elements present in the oyster are responsible for its antianemic potency and, second, that the effectiveness of the oyster in nutritional anemia can be accounted for on the basis of its Fe, Cu, and Mn content.

The question whether or not Mn is necessary and aids Cu in supplementing Fe cannot be answered from the results of these experiments.

Addendum

After this paper had been submitted to the editors, an abstract by Whipple and Wolf appeared (Amer. Jour. Physiol., Proc. Amer. Physiol. Soc., 1931, XCVII, 569) in which oysters are reported to have marked curative effects in milk anemia in rats. No results were reported as to the mineral composition of the oysters used.

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THE UTILIZATION OF THE IRON OF PROTEIN FOODS BY THE ALBINO RAT

(A) A COMPARISON OF THE GROWTH AND THE IRON ASSIMILATION AS AFFECTED BY DIFFERENT PROTEIN FOODS: (B) A COMPARISON OF PROTEIN FOODS SUPPLEMENTARY TO MILK AS SOURCES OF IRON IN NUTRITION*

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Introduction

THIS paper reports results of a continuation of a study by R. C. Miller, E. B. Forbes, and C. V. Smythe, which was discussed in an early number of this journal (27), the present investigation serving to extend the scope of the inquiry, and to review the earlier studies, by repetition, with the advantage of more critical procedures than were employed in the earlier work.

The general subject of the inquiry, namely "the development of evidence as to the specific values of meats, in comparison with other protein foods, as sources of iron in nutrition" was the same as in the earlier study, as also was the distinctive feature of basing conclusions as to the values of the diets as sources of iron upon determinations of the total iron content of the bodies of the experimental subjects. In the earlier study these iron estimations, however, were related only to the fresh weight, while in the studies here discussed they were related also to the dry weight and to the fat-and-water-free weight.

In the earlier study the protein foods compared were milk, muscle, kidney, brain, liver, beans, eggs, and peanuts; and in the present study this list was extended by the inclusion of pecans and English walnuts.

As in the earlier study these foods were used, in one series of tests, as sole sources of protein and iron, and a new series of tests was conducted in which the protein foods of interest were used not as sole sources of protein and iron but as supplements to milk, for these purposes.

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In the earlier as well as in the present work the rats were protected from vitamin deficiencies by the administration of appropriate vitamin-containing supplements; from energy deficiency by the employment of lactose, starch, and hydrogenated vegetable fat[1]; and from calcium deficiency by the inclusion of calcium carbonate in the rations which contained no milk, —thus determining that the protein foods would be compared only as sources of protein and iron, without confusion with other factors.

Important studies have been published on comparisons of the protein values of foods; but none, so far as the writers are aware, except their own earlier work, from the two-fold point of view of the present study.

The complication of the problem by the combination of the protein and the iron factors renders it difficult to draw conclusions, but this illogical procedure may be justified as necessarily incident to the consideration of valuable, associated attributes of natural foodstuffs.

The comparisons of protein values of foods, to which allusion has been made, will be briefly reviewed as contributions to the general background of information relating to the subject of interest.

Osborne and Mendel (1, 2) demonstrated that beef skeletal muscle and pig's heart, kidney and liver were suitable and adequate as sources of protein for nutrition, when other essential ingredients were present in the diet in sufficient amounts.

McCollum, Simmonds and Parsons (3, 4, 5) also studied the proteins of meats, and concluded that the proteins of beef kidney, liver and muscle, when serving as sole sources of protein, and when properly supplemented with all necessary factors other than protein, possessed about the same biological value as the proteins of the wheat kernel,—kidney, however, having a higher biological value than the other meats. When fed at high planes of intake these tissues gave no evidence of toxicity. The proteins of each were of about equal value as supplements to the proteins of cereals, and were superior to the proteins of milk in supplementing the proteins of certain seeds and of the potato. The deficiency of meats in calcium was recognized.

Mitchell and associates (6, 7, 8, 9, 10), using a modification of the Thomas method, determined the so-called biological value of the nitrogen of certain protein foods, including meats. The nitrogen of beef heart, kidney and liver was found to be utilizable to about the same extent, the biological values being 74 for heart, and 77 for kidney and liver. A value of 74 was determined for pork muscle, and 69 for beef muscle. Later work (11) indicated that a high content of connective tissue in beef made it less valuable as a source of protein. The biological values determined for meat foods were lower than those determined for milk, i.e. 83 to 86, and for eggs, i.e. 93, but higher than that for navy beans, i.e. 38.

Mitchell and Beadles (12), using the paired feeding method, found that the proteins of lean beef were not deficient in cystine, for the growth of rats, but concluded that the proteins of navy beans and milk were deficient in this amino acid.

Hoagland and Snider (13) studied the relative values, for maintenance and growth, of the protein of voluntary muscle, heart muscle, liver and kidney, from cattle, sheep and swine, using a method based on that of Osborne, Mendel and Ferry (14), in which is determined the relation between the nitrogen intake and the growth of young rats for periods of 30 and 60 days.

Pork liver fed for 30 days at a 10 per cent level of intake gave a somewhat higher value than

[1] Crisco.

did the other tissues, and beef liver a somewhat lower value, but these differences were less marked in periods of 60 days.

Pork heart and kidney, fed for periods of either 30 or 60 days at a 12.5 per cent level, gave higher values than did the other tissues. There were no outstanding differences among any of the tissues when all were fed at a 15 per cent level.

The authors concluded that in diets containing adequate quantities of protein, these tissues have approximately the same value for maintenance and growth.

Slonaker and Card (15, 16) made a study on rats restricted to a diet derived largely from plant sources, compared with others on a similar diet supplemented with meat protein. In general the rats which received meat excelled those on the restricted diet, in growth, reproduction, and duration of life.

Hitchcock (17, 18, 19) reported that the addition of meat to a well balanced diet already containing adequate protein increased the rate of growth of rats, and the efficiency of their utilization of food, and also improved the performance of rats in reproduction.

MacLeod (20) published preliminary experiments showing that an increase from 10 grams to 40 grams of fresh meat per rat per week improved reproduction and lactation. Dried meat was less favorable in its effect on reproduction than was fresh meat, and an increase in the proportion of dried meat in the ration from 3 per cent to 12 per cent seemed to favor reproduction and lactation, though the evidence was slight in extent. The increase in the milk in the basal diet from 12 per cent to 16 per cent allowed successful reproduction on both 3 per cent and 12 per cent of meat, the greater number of young being reared on the diet with 12 per cent meat.

Clayton (21, 22) found it difficult to evaluate for reproduction and lactation, the proteins of kidney, liver, milk and eggs, because of apparent vitamin deficiencies.

Nelson and associates (23, 24, 25) secured excellent growth and reproduction, but poor lactation, with rats consuming a meat ration supposedly fortified with vitamins, containing lean beef muscle as the main protein constituent.

Robscheit-Robbins (26) has reviewed the literature relating to meats, iron and other factors as affecting hemoglobin regeneration, and though the present evidence on this subject is not entirely clear and harmonious the relatively high value of animal tissues, liver and kidney in particular, for hemoglobin building, is obvious; and iron receives continued consideration as of value in the nourishment of the blood.

The general effect of these observations is to represent meats as efficient and non-toxic sources of protein in nutrition; with high supplementing value for the protein of certain vegetable foods; with favorable influences in growth, blood building, reproduction and lactation; with biologic values lower than those of milk and eggs; but with superiority to milk and to beans as sources of cystin.

Preliminary work at this Institute (27) showed in part (a) that the iron content of the bodies of albino rats was apparently normal when they had been fed diets in which meat foods constituted the sole source of protein, (b) that low iron content of the rats' bodies resulted from the feeding of diets in which the protein was supplied by hen's eggs, peanuts, beans or whole cow's milk, and (c) that all of the protein foods which were studied seemed to be superior to milk as sources of dietary iron.

It was possible, however, to question the apparent specific effects of the protein foods studied, since the gains in body weight, with the several

treatments, were very different, and since the nature of these gains, for instance, as to fat and water contents, was not determined. The investigation here discussed was conducted to clear up this uncertainty and to extend the evidence on all aspects of the problem.

The present report deals with two series of feeding experiments with rats, Series 1 serving to compare beef muscle, beef liver, beef kidney, peanuts, eggs, pecans and English walnuts, as supplementary sources of iron, when added in quantities supplying equal amounts of protein, to diets in which approximately the entire protein requirement was already supplied by skimmed milk powder. Conclusions were based on increase in body weight, and on determinations of the dry matter, the fat-free dry matter, and the iron of the bodies of the rats.

Series 2 was a continuation of the previous study (27), and compares meat and other protein foods as the sole sources of iron and protein. The general plan and scope of the experiments is outlined in Table I.

Experimental Procedure

The methods of preparation of the protein foods, except for the method of grinding, were the same as those previously employed (27). In both series the fresh meats were ground in a phosphor-bronze food chopper, which was nearly iron free, and dried in a Freas oven at about 85° C. In Series 1 the dried meats and the other protein foods were further ground in the above mentioned phosphor-bronze food chopper, but those used in Series 2 were ground in a porcelain mortar. The beans were cooked in water until tender. The water was then evaporated off, and the extract dried with the beans. The eggs were beaten and then dried. The peanuts were roasted, and the pecans and English walnuts were fed in the fresh state.

The experimental subjects were albino rats. They were fed individually and caged in such manner that they did not have access to their excreta.

The plan of feeding was the same as that previously used (27) except that equal quantities of feed were given daily, throughout an experimental week. The vitamin supplements were fed separately, Harris's yeast vitamin being administered as pellets, in the feed cup, and cod liver oil was given by mouth with a medicine dropper.

The rats were weighed at weekly intervals, and the quantity of feed to be given was computed on the basis of these weekly weights, seven daily portions being weighed at one time, and stored in tin boxes.

The quantity to be fed was determined by the expected consumption of the lot which received the least palatable feed, and the quantity of feed

TABLE I

PLAN OF EXPERIMENTATION

Series No.	Diet No.	No. of rats	Duration of experiment	Dietary sources of protein	Protein content of diets	
					Per cent	
1	23	5	15 weeks	Skimmed milk powder	15	In these diets the protein required for growth was supplied by milk powder. The other foods supplied iron and additional protein.
	24	5	"	" " " and dried beef muscle	27	
	25	5	"	" " " " " " liver	"	
	26	4	"	" " " " peanuts	"	
	27	5	"	" " " " dried eggs	"	
	28	5	"	" " " " dried beef kidney	"	
	30	4	"	Skimmed milk powder	15	
	31	4	"	" " " and pecans	21	
	32	4	"	" " " " English walnuts	21	
2	33	5	15 weeks	Skimmed milk powder	16	In these diets the protein foods served as sole source of protein and of iron.
	34	5*	"	Dried beef brain	"	
	35	4	"	" " muscle	"	
	36	5	"	" " kidney	"	
	37	5	"	" " liver	"	
	38	5	"	" cooked beans	"	
	39	5	"	" eggs		
	40	4	"	Peanuts		

* Two members of this group died in the course of the experiment.

given to this lot, in relation to its live weight, constituted the basis for the allowance of feed to each other lot. The quantities of food given, on this basis, are presented for Series 1 in Table II, and for Series 2 in Table V. Experience gained with this method of feeding in the experiments of Series 1 made possible the more successful regulation of the food intake in Series 2. Such variations as occurred were caused mainly by the inability of the experimenters to foretell the exact quantity of food that would be consumed. A decrease in the rate of feed allowance in the experiments of Series 2 lessened this difficulty.

When this method of feed control is used the apparent palatability of the diet, which serves to determine the rate of feeding, may be fundamental, in the sense of being truly characteristic, or may be a secondary result of nutritive deficiency and consequent low state of nutrition. In this investigation the quantity of food consumed in proportion to the body weight became smaller each week until the maximum body weight was attained—as naturally prevails during growth.

Also, by this method of feeding, animals which do not grow well are restricted in food intake in comparison with animals whose growth is good, in consequence of the fact that although the quantities of food offered are in the same proportion to the body weights, in both cases, the maintenance requirement of energy producing nutriment is relatively smaller for the larger animal, thus giving it a larger quantity of food available for body increase than the smaller animal receives, in relation to body weight. This situation tends to exaggerate differences in nutritive values of foods— the greater the differences, the greater the exaggeration of these differences.

The experimental feeding periods were fifteen weeks in length, after which the rats were killed by means of chloroform. Their bodies were prepared for analysis by the removal and discarding of the alimentary tracts, and thereafter by drying for 24 hours, at 100° C., in a Freas oven. The bodies were then crushed with a pestle, and further dried over sulfuric acid in vacuum desiccators for periods up to 120 days in length. By this time the rats' bodies reached almost constant weights, but in no case did they seem to become absolutely dry. The drying was discontinued when the losses in weight had become practically negligible. The bodies were then broken up, transferred to paper capsules, and extracted with ether for 72 hours in large Soxhlet extractors. The fat obtained from this extraction was weighed; the dry, fat-free bodies were ashed in a muffle furnace; and iron was determined in the residues.

Iron was determined by the modified thiocyanate-acetone method previously used (27), with the additional modification suggested by Elvehjem

TABLE II

SERIES 1. AVERAGE NUMBER OF GRAMS OF FOOD EATEN PER WEEK, PER GRAM OF BODY WEIGHT

Diet No.	Source of protein	Week No.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
23	Skimmed milk powder	.74	.53	.51	.50	.52	.42	.39	.37	.31	.32	.29	.29	.27	.25	.25
24	Skimmed milk powder and beef muscle	.71	.67	.61	.51	.48	.44	.40	.39	.36	.30	.28	.29	.25	.25	.25
25	Skimmed milk powder and beef liver	.55	.75	.60	.48	.52	.50	.47	.47	.37	.31	.30	.29	.27	.25	.25
26	Skimmed milk powder and peanuts	.67	.56	.52	.48	.44	.40	.31	.31	.24	.31	.29	.27	.28	.24	.25
27	Skimmed milk powder and dried eggs	.93	.58	.51	.51	.49	.43	.39	.37	.32	.31	.31	.29	.27	.25	.25
28	Skimmed milk powder and beef kidney	.70	.60	.51	.51	.49	.42	.39	.37	.32	.31	.30	.29	.27	.20	.25
30	Skimmed milk powder (high fat)	.62	.42	.40	.45	.46	.37	.35	.35	.31	.28	.28	.27	.26	.21	.23
31	Skimmed milk powder and pecans	.55	.65	.48	.48	.43	.33	.31	.31	.27	.24	.23	.22	.21	.21	.19
32	Skimmed milk powder and English walnuts	.64	.63	.50	.46	.43	.35	.34	.28	.26	.26	.25	.21	.23	.21	.18

(28), whereby any pyrophosphate present is converted to orthophosphate, by neutralizing the hydrochloric acid solution of the ash with dilute sodium hydroxide, and boiling for about an hour. Slightly higher results were obtained in some cases when this procedure was used, and it was therefore followed throughout these experiments. Extraction of the colored ferric thiocyanate with amyl alcohol yielded results consistently slightly lower than those obtained with the procedure employing the acetone.

Experimental Series 1

The composition of the diets fed in Series 1 is given in Table III. The proportion of skimmed milk powder in each diet was such as to supply approximately the amount of protein required for maintenance and for growth, the other protein foods being included in proportions furnishing

TABLE III

SERIES 1. COMPOSITION OF DIETS. (PARTS PER 100)*

Diet No.	23	24	25	26	27	28	30	31	32
Skimmed milk powder	44.37	44.37	44.37	44.37	44.37	44.37	44.37	44.37	44.37
Dried beef muscle	—	20.00	—	—	—	—	—	—	—
Dried beef liver	—	—	22.00	—	—	—	—	—	—
Peanuts	—	—	—	53.07	—	—	—	—	—
Dried eggs	—	—	—	—	33.70	—	—	—	—
Dried beef kidney	—	—	—	—	—	20.90	—	—	—
Pecans	—	—	—	—	—	—	—	50.00	—
English walnuts	—	—	—	—	—	—	—	—	39.40
Lactose	10.00	10.00	10.00	2.06	10.00	10.00	—	—	—
Starch	31.95	21.03	15.45	—	10.52	23.08	19.28	5.13	6.20
Crisco	13.18	4.10	7.68	—	.91	1.15	35.85	—	9.53
NaCl	.50	.50	.50	.50	.50	.50	.50	.50	.50
Iron content (per cent)	.0010	.0020	.0051	.0017	.0037	.0049	.0010	.0020	.0027

* Cod liver oil and Harris Yeast Vitamin were fed daily with each diet.

equal quantities of supplementary protein. The energy contents of the diets were equalized by means of lactose, starch and hydrogenated vegetable fat[2]. Lactose was used in place of the sucrose previously employed with the idea that it might be less readily fermentable in the intestine, but neither sucrose nor lactose seemed to be any more or less desirable than starch alone, and the latter was used by itself, in Series 2, as a source of carbohydrate.

[2] Crisco.

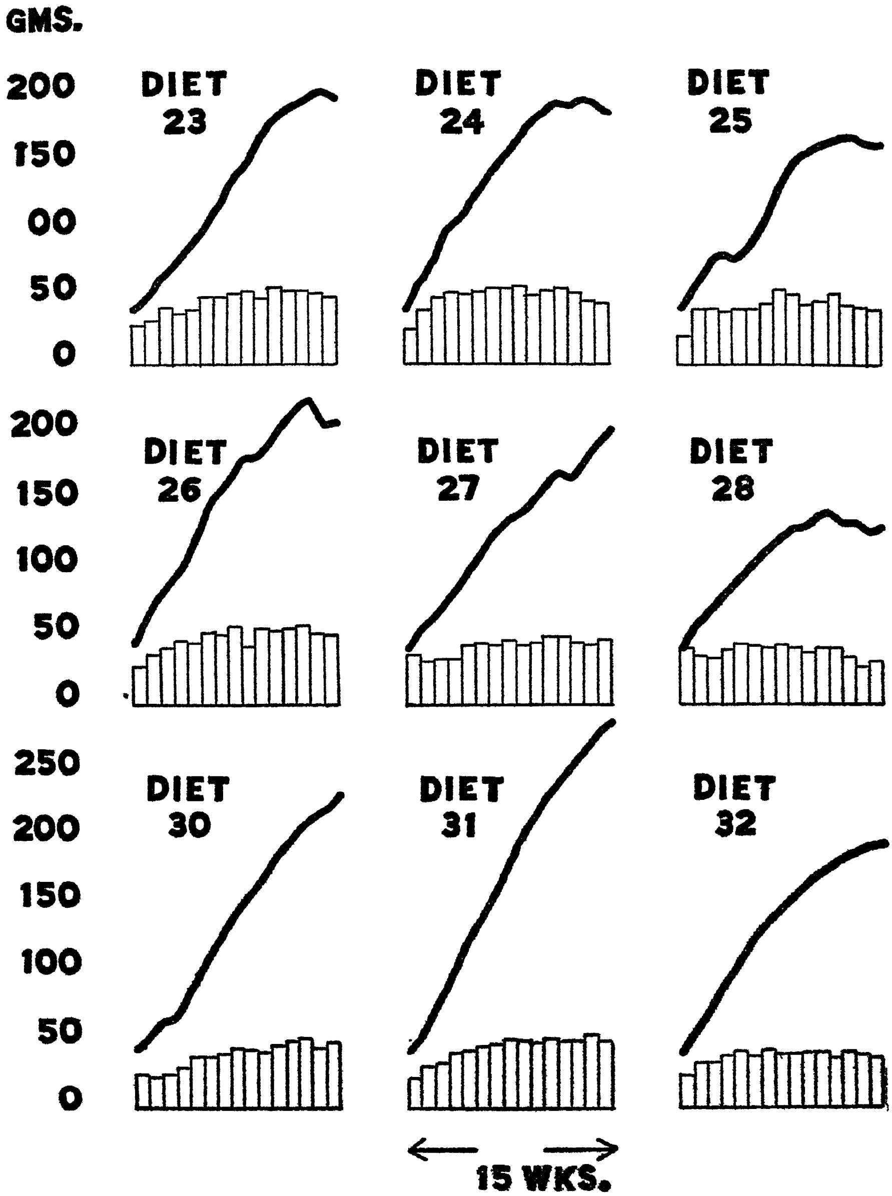

CHART 1. The curves of average growth and the food intakes of rats fed various diets.

Diet No. 23, milk; 24, milk and beef muscle; 25, milk and beef liver; 26, milk and peanuts; 27, milk and eggs; 28, milk and beef kidney; 30, milk; 31, milk and pecans; 32, milk and English walnuts.

It was not possible to supply the same proportions of protein and energy in the diets by means of English walnuts and pecans, as by the other protein foods—because of their high fat and relatively low protein contents. Diets Nos. 31 and 32, therefore, contained less protein and more fat than did diets Nos. 23 to 28; and diet No. 30 was designed to be the same as diet No. 23, except that to permit of comparison with diets Nos. 31 and 32 its fat and energy contents were made the same as in these diets.

Curves representing average growth, and histographs of the total weekly food consumption are presented in Chart No. 1; and data with respect to body gain, food and iron intake, and composition of the bodies of the rats are given in Table IV.

In spite of the restricted food intake, at least good average growth was obtained with all of the rats of Series 1 except those groups which received milk powder and beef liver, and milk powder and beef kidney. The feces of both of these groups were watery, and obviously indicated indigestion. This abnormal condition was overcome naturally during the first weeks in the group fed liver, but lasted throughout the entire feeding period for the kidney group and probably accounted, at least in part, for their poor growth performance.

The group fed milk powder and pecans made remarkably good growth which cannot be critically explained by the results of the present work. A marked superiority of the proteins of the pecan to those of the English walnut in supplementing the proteins of milk is apparent. Cajori (29, 30) reported normal growth in rats on diets in which English walnuts or pecans constituted the sole source of protein, but he concluded that it was necessary first to remove the tannin of the pecans. In the course of the experiments here discussed no indications of unfavorable effects of any sort from the feeding of the pecans were observed. Beef muscle, peanuts and eggs are here shown to be of about equal value as protein supplements to skimmed milk powder.

Table IV shows that the growth of different groups was not in proportion to the food intake, and must have been influenced, therefore, to a considerable extent, by the quality of the various diets. Among the groups which received diets Nos. 23 to 27, inclusive, the rats fed milk powder and eggs made the most efficient utilization of food; the groups fed milk powder alone, or milk powder and muscle, liver, or peanuts, being about equally efficient, while the rats fed milk powder and kidney were the least efficient in their utilization of food for growth. It should be borne in mind, in relation to these observations, that the food consumption for each experimental lot was determined, in large measure, by its growth performance.

The composition of the rats, with respect to dry matter and ether extract, reveals the fact that among the rats which grew well, there is not a wide variation in the body content of these substances. It is also true that where good growth occurred the body content of dry matter and fat is relatively high. In the case of the rats which received diet No. 28 and which made poor growth, the dry matter and fat content of the rats were lower than in any other group of the series. In harmony with the observation which has been made by many workers in relation to other animals, when the dry matter of the body was low the fat content was also low, since fat and water tend strongly to vary reciprocally; thus the data for Series 1 give a correlation between the ether extract and dry matter content of the rats of $0.91 \pm .015$.

The iron content of the bodies of the rats in the several experimental lots is graphically represented, in Chart No. 2, on the absolute, the fresh, the dry, and the fat-free dry bases, with accompanying representation of the dry matter and of the ether extract.

The representation on the fat-free dry basis most clearly and significantly shows the superiority of the meats, fed supplementary to milk, and the inferiority of milk, as sources of iron; with eggs, peanuts, walnuts and pecans, all fed supplementary to milk, occupying intermediate positions in this regard.

On the dry basis the iron content of the rats which received kidney was very high, as a result of the poor growth which these rats made, and the low fat content of their bodies; and on the absolute basis the iron content of the rats which received pecans was high because of the remarkable growth made from this diet. It is thus apparent that it was by no means only the iron content of the diets which determined the iron content of the bodies of the rats.

The percentage variations in the iron content of the body may be regarded as determined, in part, by the iron enrichment or impoverishment of the living substance, and in part as arithmetical effects of variations in the fat and water contents—which are to a large extent reciprocal.

The physiological significance of these variations is not revealed by the method of these experiments, which merely establishes suggestive facts that can be interpreted only by intensive physiological investigation.

Experimental Series 2

The composition of the diets fed in Series 2 is given in Table V. In this series the protein foods constituted the sole sources of protein and iron in the diets, and were included in such amounts as supply equal quantities

TABLE IV

SERIES 1. FOODS CONSUMED, CAINS IN BODY WEIGHT, AND COMPOSITION OF BODIES OF RATS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. weekly food intake per gram of live weight	Av. daily iron intake	Dry matter content of body minus alimentary tract	Ether extract content of body minus alimentary tract	Iron content of body minus alimentary tract			
									Total	Per cent of fresh wt.	Per cent of dry, fat free wt.	Per cent of dry wt.
			grams	grams	grams	mgs.	per cent	per cent	mgs.	per cent	per cent	per cent
516	23	Skimmed	1.68	7.17	0.43	0.07	36.74	11.87	6.60	.0032	.013	.009
517	23	milk	1.63	7.83	0.46	0.08	41.35	17.25	4.93	.0024	.010	.006
518	23	powder	.94	5.76	0.41	0.06	38.20	12.74	4.50	.0034	.014	.009
519	23		1.12	5.71	0.40	0.06	42.42	18.07	5.02	.0034	.014	.008
520	23		1.94	8.12	0.41	0.08	39.53	15.21	6.13	.0026	.011	.007
		Average	1.46	6.92	0.42	0.07	39.65	15.03	5.44	.0030	.012	.008
521	24	Skimmed	1.00	6.21	0.42	0.12	44.44	19.92	7.41	.0054	.022	.012
522	24	milk	2.00	9.15	0.40	0.18	38.95	12.82	9.65	.0041	.016	.011
523	24	powder	1.33	7.70	0.44	0.15	39.67	13.42	7.62	.0044	.017	.011
524	24	and beef	1.59	7.81	0.41	0.16	43.29	18.95	8.91	.0052	.019	.010
525	24	muscle	1.02	5.90	0.41	0.12	43.18	18.84	7.07	.0053	.022	.012
		Average	1.39	7.35	0.42	0.15	41.91	16.79	8.13	.0049	.019	.011

526	25	Skimmed	1.87	7.50	0.42	0.38	37.59	11.39	8.86	.0043	.016	.011
527	25	milk	1.02	5.82	0.43	0.30	39.74	14.32	6.58	.0051	.020	.013
528	25	powder	1.01	6.30	0.44	0.32	38.92	12.54	7.03	.0054	.021	.014
529	25	and beef	1.06	5.89	0.43	0.30	37.94	11.43	7.36	.0056	.021	.015
530	25	liver	1.23	6.03	0.41	0.31	39.84	14.61	7.19	.0047	.019	.012
		Average	1.24	6.31	0.43	0.32	38.81	12.86	7.40	.0050	.019	.013
546	28	Skimmed	.87	5.88	0.40	0.29	31.68	3.39	6.58	.0052	.018	.016
547	28	milk	.94	6.01	0.39	0.29	31.78	3.47	6.94	.0052	.019	.017
548	28	powder	.94	5.64	0.39	0.28	34.51	7.80	5.99	.0045	.017	.013
549	28	and beef kidney	.77	4.70	0.40	0.24	31.69	4.27	5.22	.0049	.018	.015
		Average	.88	5.56	0.40	0.28	32.42	4.73	6.18	.0050	.018	.015
531	26	Skimmed	1.10	6.11	0.39	0.10	43.26	19.42	6.22	.0042	.018	.010
532	26	milk	1.17	6.04	0.36	0.10	42.23	18.15	5.58	.0035	.015	.008
533	26	powder	1.80	7.90	0.38	0.13	39.80	15.14	7.84	.0035	.014	.009
534	26	and	1.67	7.32	0.38	0.12	40.31	15.38	5.60	.0028	.011	.007
535	26	peanuts	1.83	7.92	0.37	0.14	39.41	15.08	6.46	.0029	.012	.007
		Average	1.51	7.06	0.38	0.12	41.00	16.63	6.34	.0034	.014	.008
541	27	Skimmed	1.88	6.76	0.42	0.25	37.65	12.23	7.65	.0035	.014	.009
542	27	milk	1.21	5.82	0.42	0.21	43.35	19.33	3.59	.0023	.010	.005
543	27	powder	1.19	5.51	0.41	0.20	37.06	11.14	7.72	.0051	.020	.014
544	27	and	1.30	5.83	0.41	0.22	34.81	9.89	6.33	.0038	.014	.011
545	27	eggs	2.15	7.23	0.41	0.27	37.68	12.83	8.59	.0035	.014	.008
		Average	1.55	6.23	0.41	0.23	38.11	13.08	6.78	.0036	.014	.009

TABLE IV
SERIES 1. (*continued*)

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. weekly food intake per gram of live weight	Av. daily iron intake	Dry matter content of body minus alimentary tract	Ether extract content of body minus alimentary tract	Iron content of body minus alimentary tract			
									Total	Per cent of fresh wt.	Per cent of dry, fat free wt.	Per cent of dry wt.
			grams	grams	grams	mgs.	per cent	per cent	mgs.	per cent	per cent	per cent
565	30	Skimmed	1.91	5.63	0.35	0.06	38.64	15.63	7.49	.0033	.014	.009
566	30	milk	1.50	5.73	0.34	0.06	40.02	16.78	3.61	.0020	.008	.005
567	30	powder	1.40	5.32	0.35	0.05	42.41	19.58	6.04	.0034	.015	.008
568	30	(high fat)	2.38	6.70	0.35	0.07	41.46	19.71	5.94	.0021	.010	.005
		Average	1.80	5.85	0.35	0.06	40.63	17.93	5.77	.0027	.012	.007
560	31	Skimmed	2.70	7.19	0.34	0.14	40.16	16.64	8.79	.0029	.013	.007
561	31	milk	1.62	5.17	0.31	0.10	41.97	18.74	6.12	.0032	.014	.008
562	31	powder	2.53	6.71	0.34	0.13	41.82	18.29	8.18	.0029	.012	.007
563	31	and pecans	2.57	7.26	0.34	0.15	38.98	14.56	7.88	.0028	.011	.007
		Average	2.36	6.58	0.33	0.13	40.73	17.06	7.74	.0030	.013	.007
570	32	Skimmed	1.48	5.39	0.33	0.15	37.66	13.16	5.33	.0029	.012	.008
571	32	milk	1.60	5.71	0.33	0.15	38.94	14.63	6.59	.0034	.014	.009
572	32	powder	1.44	5.65	0.36	0.15	40.89	17.38	4.82	.0027	.011	.007
573	32	and English walnuts	1.45	5.81	0.37	0.16	39.24	14.72	5.99	.0033	.014	.009
		Average	1.49	5.64	0.35	0.15	39.18	14.97	5.68	.0031	.013	.008

of protein. The energy contents of the diets were also equalized by varying the proportions of starch and hydrogenated vegetable fat. Diet No. 40 necessarily had a higher fat content and a higher energy value than did the other diets of this series, on account of the relatively high fat and low protein contents of peanuts.

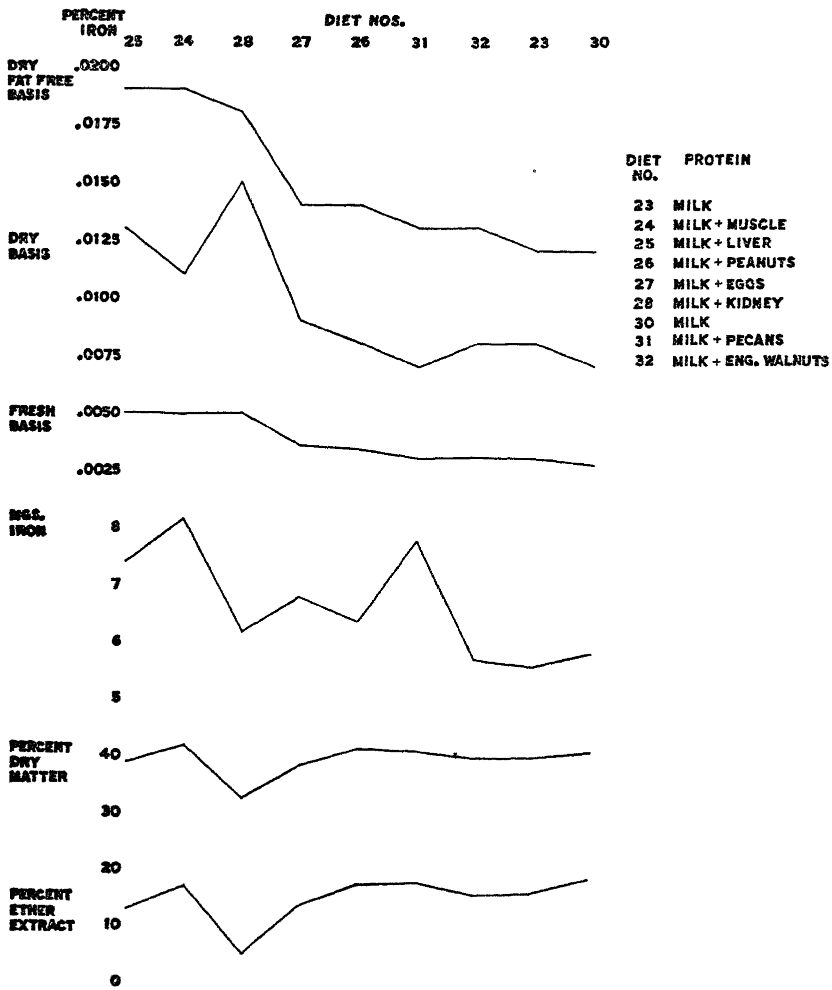

CHART 2.—The iron, dry matter and ether extract contents of the bodies of rats fed various diets.

The curves of average growth and the average gross food intakes are represented in graph No. 2, and data with respect to body gains, food intake, and the composition of the bodies of the rats are given in Table VI.

TABLE V

SERIES 2. AVERAGE NUMBER OF GRAMS OF FOOD EATEN PER WEEK, PER GRAM OF BODY WEIGHT

Diet No.	Source of protein and iron	Week No.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
33	Skimmed milk powder	.60	.55	.45	.41	.38	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
34	Beef brain	.58	.55	.45	.41	.37	.36	.35	.33	.29	.27	.27	.25	.24	.24	.24
35	Beef muscle	.60	.55	.45	.42	.37	.36	.35	.33	.29	.28	.26	.25	.24	.24	.24
36	Beef kidney	.60	.55	.45	.41	.37	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
37	Beef liver	.60	.55	.46	.41	.37	.36	.35	.33	.29	.27	.24	.25	.24	.24	.24
38	Beans	.53	.54	.45	.41	.37	.36	.35	.33	.29	.28	.26	.25	.24	.24	.24
39	Eggs	.60	.55	.45	.41	.36	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24
40	Peanuts	.60	.55	.45	.41	.37	.36	.35	.33	.29	.27	.26	.25	.24	.24	.24

The best growth was obtained with the diet in which milk powder served as the sole source of protein, but good growth occurred with the rats which received beef muscle, beef kidney, beef liver, or eggs. Poor growth resulted with beef brain, beans, and peanuts, and two of the rats which received beef brain died early in the experiment. The comparative growth of the lots of rats in this series was doubtless influenced, in a manner tending to

TABLE VI

SERIES 2. COMPOSITION OF DIETS (PARTS PER 100)*

Diet; No. →	33	34	35	36	37	38	39	40
Skimmed milk powder	48.19	—	—	—	—	—	—	—
Dried beef brain	—	31.88	—	—	—	—	—	—
" " muscle	—	—	24.08	—	—	—	—	—
" " kidney	—	—	—	22.06	—	—	—	—
" " liver	—	—	—	—	22.65	—	—	—
" beans	—	—	—	—	—	64.83	—	—
" eggs	—	—	—	—	—	—	32.72	—
Peanuts	—	—	—	—	—	—	—	52.33
Corn starch	40.31	66.27	66.43	64.46	63.64	19.88	63.49	46.17
Hydrog. veg. fat	10.00	0.35	7.99	11.98	12.21	13.79	2.29	—
NaCl	.50	0.50	.50	.50	.50	.50	.50	.50
$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Iron content (per cent)	.0015	.0041	.0032	.0066	.0061	.0059	.0034	.0021

* Cod liver oil and Harris Yeast Vitamin were fed daily with each diet.

obscure the results sought, by the poor food consumption of the lot which received brain, since this lot determined and restricted the food allowance to all other lots. Under these conditions it is natural that the growth of the several lots of rats was rather closely in accord with the quantity of food allowed.

The poor growth of the lots which received beans, brain, and peanuts, and the wide variability of the individual performance within these lots, call attention to the unsatisfactory quality of bean, brain, and peanut protein as sole sources of this component of a diet.

In comparison with the rats in Series 1, which made more extensive growth, the rats in this series were characterized by slightly lower contents of dry substance, these values being lowest in the two groups (which received the bean and the brain diets) which made the poorest growth.

The growth produced by these diets is in harmony with the conclusions of Mitchell (10) that milk protein has a higher biological value than meat

protein, and that the biological values of the proteins of liver, kidney, and muscle are about equal.

The lot which received milk performed in a manner representative of the established character of this food as a poor source of iron in nutrition, the iron content of this lot of rats, on the percentage basis, being decidedly the lowest of all.

Among the lots which received liver, kidney, muscle, and eggs—the individual variation was such that it is not possible to distinguish between them with certainty. On the basis of this evidence liver, kidney, muscle, and eggs appear to be good—and nearly equally good—sources of food iron.

The absolute iron content of the bodies of the rats was highest (7 mg.) for those which received liver, was slightly lower (6.53 mgs. and 6.43 mgs.) for those which received muscle and kidney, and slightly lower than these (6.13 mgs.) for the rats which received eggs. The groups receiving beans, milk powder, brain and peanuts contained from 4.82 to 4.05 mgs. of iron, the values decreasing in the order in which the protein foods are named.

When the iron contents of the bodies are expressed as percentages of the fresh or the dry weights, the values for the milk, muscle, kidney and liver groups are in good agreement with those of the same groups in the previous work of this Institute (27); and although not perfectly comparable, they are of the same order of magnitude as the groups receiving the same foods as protein and iron supplements in Series 1 of the present paper.

The percentages of iron for the liver, muscle and kidneys groups agree closely among themselves, and are considerably higher than those for the milk group. The figures for the iron content of the milk group are but slightly higher than those reported by Supplee and associates (31) for rats receiving liquid whole milk and reconstituted dry milk. Eggs are here shown to better advantage than in the earlier work (27), and must be grouped with the meat foods as a source of dietary iron, whereas previously they appeared to have a value as a source of iron intermediate between meats and milk.

The rats which received the brain, bean, and peanut diets had percentage iron contents as high as or higher than had the meat rats. In these cases the high iron contents did not signify extensive storage of iron but were due to poor growth and to poor fattening (as indicated by low dry matter contents), the food iron therefore being concentrated as the result of the restriction of those processes which normally dilute or extend it. This is most clearly seen in the case of rat No. 582, which made the poorest growth of all. Its body content of dry matter is the lowest of the series and its percentage content of iron, on either the fresh or the dry basis, is

the highest of all. The relatively high percentages of iron in the bodies of the rats which received brain, beans, and peanuts, therefore, when considered together with the poor growth of these rats, can not be interpreted as indicating superiority of these foods as sources of food iron.

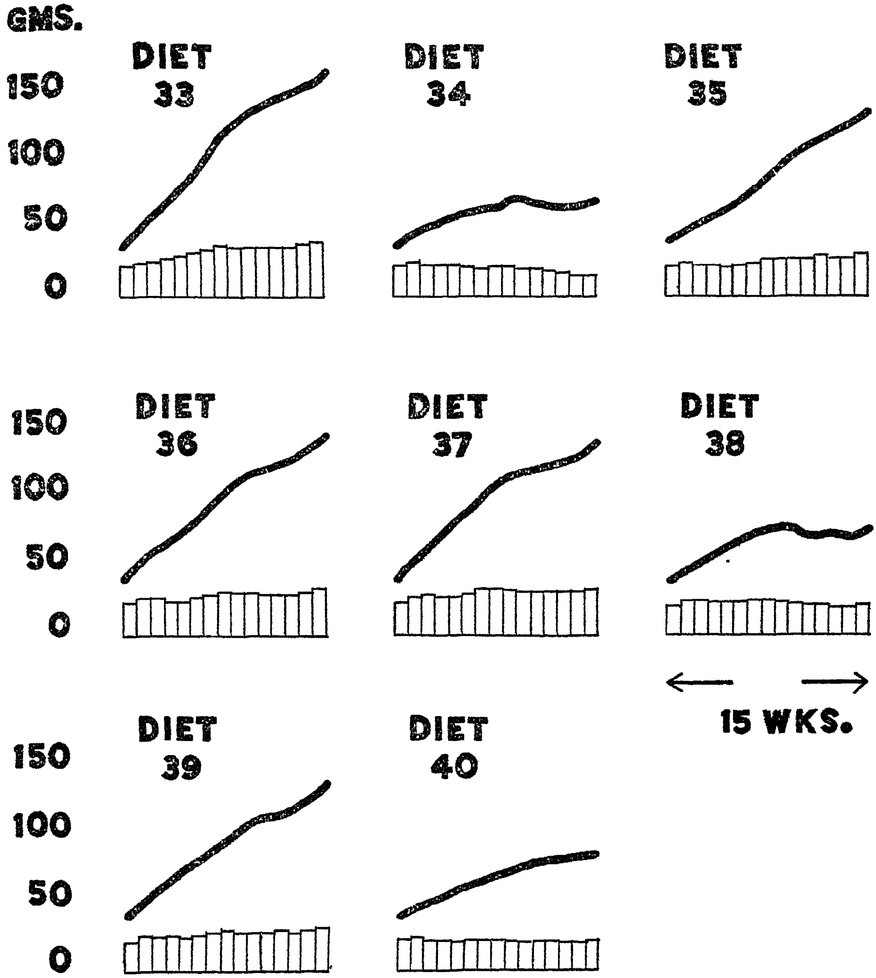

CHART 3.—The curves of average growth and the food intakes of rats fed various diets. Diet No. 33, milk as source of protein; 34, beef brain; 35, beef muscle; 36, beef kidney; 37, beef liver; 38, beans; 39, eggs; 40, peanuts.

The results of Series 2, therefore, indicate that as sole sources of food iron beef muscle, kidney, liver and eggs are superior to milk; and that beef

TABLE VII

SERIES 2. FOODS CONSUMED, GAINS IN BODY WEIGHT, AND COMPOSITION OF BODIES OF RATS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. weekly food intake per gram of live weight	Av. daily iron intake	Av. daily food intake	Dry matter content of body minus alimentary tract	Iron content of body minus alimentary tract		
								Total	Per cent of fresh weight	Per cent of dry weight
			grams	grams	mgs.	grams	per cent	mgs.	per cent	per cent
574	33	Skimmed milk powder	.92	.35	.06	4.03	36.24	3.80	.0030	.008
575			1.16	.35	.06	4.32	32.38	4.34	.0029	.009
576			.90	.35	.06	4.20	36.05	5.05	.0041	.011
577			1.53	.35	.08	5.14	34.11	4.91	.0026	.008
578			1.74	.35	.08	5.60	33.82	6.17	.0030	.009
	Average		1.25	.35	.07	4.66	34.52	4.85	.0031	.009
581	34	Beef brain	.46	.35	.13	3.27	36.70	4.47	.0055	.015
582			.11	.34	.11	2.68	28.59	3.88	.0084	.029
583			.30	.35	.13	3.14	30.42	4.29	.0064	.021
	Average		.29	.35	.12	3.03	31.90	4.21	.0068	.022
585	35	Beef muscle	.87	.35	.11	3.42	34.31	6.56	.0054	.016
586			1.00	.35	.13	3.98	34.88	6.76	.0048	.014
587			1.01	.35	.13	4.10	34.93	6.49	.0045	.013
588			.89	.35	.12	3.85	35.82	6.30	.0050	.014
	Average		.94	.35	.12	3.84	34.99	6.53	.0049	.014
589	36	Beef kidney	.83	.35	.26	3.95	38.32	6.22	.0052	.013
590			1.05	.35	.27	4.15	35.53	6.02	.0041	.012
591			1.47	.35	.33	5.04	36.38	7.50	.0039	.011
592			1.10	.35	.30	4.57	36.01	6.88	.0044	.012
593			.54	.35	.26	3.88	33.19	5.51	.0058	.017
	Average		1.00	.35	.28	4.32	35.89	6.43	.0047	.013

594	37	Beef liver	.52	.35	.22	3.58	35.75	5.69	.0062	.017
595			1.09	.35	.27	4.38	35.62	6.52	.0044	.012
596			.90	.34	.26	4.25	38.02	6.88	.0053	.014
597			.91	.35	.28	4.60	39.20	7.74	.0056	.014
598			1.25	.35	.30	4.91	42.41	8.19	.0049	.012
	Average		.93	.35	.27	4.34	38.20	7.00	.0053	.014
599	38	Beans	.37	.34	.20	3.43	32.05	4.46	.0054	.017
600			.36	.35	.18	2.99	33.83	4.78	.0067	.020
601			.45	.34	.19	3.24	33.27	5.20	.0064	.019
602			.39	.34	.18	3.11	34.77	5.18	.0070	.020
603			.57	.34	.20	3.45	32.18	4.82	.0050	.015
	Average		.43	.34	.19	3.24	33.22	4.89	.0061	.018
604	39	Eggs	1.10	.35	.13	3.73	38.78	6.82	.0047	.012
605			.61	.35	.11	3.16	36.82	5.42	.0055	.015
606			1.14	.35	.14	4.14	37.14	6.28	.0041	.011
607			.69	.35	.13	3.87	36.83	5.28	.0048	.013
608			1.20	.35	.15	4.48	36.13	6.86	.0043	.012
	Average		.95	.35	.13	3.88	37.14	6.13	.0047	.013
609	40	Peanuts	.24	.35	.05	2.43	34.46	3.56	.0063	.018
610			.41	.35	.06	2.84	32.85	3.85	.0051	.016
612			.83	.35	.08	3.88	40.83	4.98	.0041	.010
613			.43	.34	.06	3.04	37.26	3.83	.0048	.013
	Average		.48	.35	.06	3.05	36.35	4.05	.0051	.014

brain, beans, and peanuts are poor sources of iron apparently because of the poor quality of their protein from a nutritional point of view.

SUMMARY

Two series of experiments were conducted to ascertain: 1.—the relative values of beef muscle, beef liver, beef kidney, peanuts, eggs, pecans and English walnuts, as sources of iron, when each food served as a source of protein supplementary to milk powder, and 2.—the relative values of milk powder, beef muscle, beef liver, beef kidney, beef brain, eggs and peanuts as sources of iron, when each served in the diet as the sole source of protein and iron.

The experimental subjects were albino rats which were fed individually for fifteen weeks.

The best growth resulted from the diet in which the sources of protein were milk powder and pecans, though excellent growth also resulted from the diet in which milk powder was the sole source of protein. Milk powder as a source of protein, when supplemented with beef muscle, eggs, peanuts and English walnuts afforded good growth, while somewhat inferior performance resulted from milk powder and liver, and milk powder and kidney.

When serving as the sole source of protein in the diet, beef muscle, beef kidney, beef liver and eggs, all supported good growth, but were excelled in this respect by milk powder. Poor growth resulted from diets deriving their protein from beef brain, beans and peanuts.

The values of protein foods fed supplementary to milk powder, as sources of iron, were stated on the basis of absolute quantities stored, and on the basis of the contents of iron in the bodies of the rats on the fresh, the dry, and the fat-free dry basis.

On the absolute basis beef muscle, beef liver, and pecans were superior sources of iron; kidney, eggs, and peanuts were of moderate value; while English walnuts and milk powder were the poorest sources of this nutrient.

On the fresh and the fat-free dry bases beef muscle, beef liver, and beef kidney were superior sources of iron; eggs and peanuts were of moderate value; while pecans, walnuts and milk appeared to be the poorest sources of iron.

The dry body weight of the rat appeared not to be a significant basis of reference for iron contents.

As sole sources of iron, reckoned on the basis of the fresh body weights, beef muscle, beef liver, beef kidney, and eggs were superior sources of iron, while milk as usual rated poorest in this regard, the status of brain, beans,

and peanuts appearing in an unfavorable light but being questionable because of the poor or irregular growth of the rats.

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THE EFFECT OF PARTIAL DEPLETION OF VITAMIN B COMPLEX UPON LEARNING ABILITY IN RATS

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THE depletion of vitamin B complex may be either partial or complete. With complete depletion either beriberi or pellagra is developed, depending on whether the depletion is more in vitamin B (B_1) or in vitamin G(B_2) respectively. With partial depletion the usual symptom of nervous derangement may not occur in adult patients; but with children, whose nervous system is still in a stage of development, partial depletion of vitamin B complex over a long period of time might be detrimental to the proper development and function of the nervous system. That beriberi and pellagra are nerve diseases due to complete depletion of vitamin B complex is known to the public, but the detrimental effect of partial depletion has so far escaped the attention even of most physicians. Although beriberi is common in the Orient, and pellagra is prevalent in the southern states, partial depletion of vitamin B complex constitutes a world wide problem with breast and artificially fed infants and older children.

For centuries beriberi has been widespread in the Orient, and is said to have been known to the Chinese as early as 2600 B.C. The Malay States, Siam, Korea, Japan and the Philippine Islands have been greatly afflicted by it and it is more or less prevalent in India and in Africa. That it is not confined to tropical regions is shown by its occurrence in recent times in Newfoundland, Labrador and Norway. Infantile beriberi—the most accentuated form of vitamin B deficiency—is produced in breast-fed infants whose mothers partake of a diet composed mainly of polished rice. Pigeons or chickens which are restricted to a diet of milled rice develop in 2 to 4 weeks a condition of paralysis strikingly suggestive of beriberi in man. The symptoms due to derangement of the nervous system are the most spectacular, leading as they do to applying the name "polyneuritis" to the whole symptom complex.

"It should not be supposed, however, that beriberi occurs only on a rice diet. It has been shown that a number of other foodstuffs are either relatively or absolutely deficient in the beriberi-preventing vitamin. Highly milled white wheat flour and various carbohydrate foods, such as tapioca, sago and the various sugars, are quite as deficient in vitamin as rice and

when used too exclusively in the diet will produce beriberi in men or fowls with great certainty." (1)

"In point of quantity consumed white flour is the most important energy food in America and Europe. It is remarkable because it is notably deficient in more dietary factors than any other single food entering into the diet, except sugars, starches and fats which are marketed in the pure state." (2)

Furthermore, "the use of much of our food in highly refined forms has become customary in this country, and the custom is strongly fostered by the manufacturers because the refined forms are relatively immune to spoilage. So long as this custom persists, vitamin B will constitute a factor of very real practical importance in food values."(3)

McCollum points out the danger to health in an adherence to a diet in which milled cereal products, particularly white bread, and sugar, syrup, tubers and meat of the muscle type predominate. This danger is shown by the alarming increase in the incidence of defective nutrition among infants and children, brought about by the poor diet of the expectant and nursing mothers and of infants and older children.

Recent work of Goldberger and others shows that certain symptoms resembling those which occur in pellagra can be definitely traced to a deficiency of vitamin $G(B_2)$. These symptoms are gastro-intestinal disturbance with diarrhea, degenerative disturbance of the nervous system, including mental changes, loss of weight, retarded growth and skin eruptions on the face, neck, hands and feet.

"During 1917 in an aggregated population of 22,653 individuals in some cotton-mill villages of South Carolina, 1,147 cases of pellagra (an incidence rate of 50.6 per 1,000) were observed. Of the 4,104 households among which that population was distributed 18.5 per cent had at least one member of each affected by the disease in that year. Pellagra (in an endemic locality) is very much (two to six times) more prevalent than the experience of the physicians of the locality would seem to indicate. The observation of age incidence appears to indicate that endemic pellagra is preponderatingly a disease of children of from 2 to 15 years of age." (4)

McCollum (5) is of the opinion that "improvement of the dietary is of the greatest significance for the welfare of a large percentage of American children of the present generation. Leete (1921) summarized the statistics of examination of a large part of the twenty million school children in the United States. She found . . . 15 to 25 per cent were suffering from malnutrition. Terman (1914) stated that about fourteen million of the twenty million school children in the United States were handicapped by some kind

of physical defect, and that not far from two million were suffering from a grave form of malnutrition."

The foregoing discussion may be summarized as follows: 1.—Depletion of vitamin B complex is etiologically related to such nerve diseases as beriberi, pellagra and polyneuritis, all of which involve changes in the nervous system; 2.—polished rice, the chief food supply in the Orient, and white flour bread, the main staple food in Europe and America, are both utterly deficient in vitamin B complex; and 3.—while infantile beriberi is common in the Orient, pellagra and partial depletion of vitamin B complex are found to be prevalent among the children of certain sections of the United States.

With this background in mind, one will immediately realize that the problem of vitamin B complex depletion is one of world-wide importance.

It is the purpose of our investigation to study the effect of partial depletion of vitamin B complex produced during the nursing period upon the higher nervous function or learning ability of the first and second generations of experimental animals.

We depleted our animals during the nursing period when the nervous system is still in the process of differentiation. If the depletion of vitamin B complex does produce any harmful effect on the nervous system at all, one would naturally expect to find the greatest amount of detriment when depletion is introduced during this early period.

White rats were used in our experiment because at their birth, like infants, they have nervous systems which are incompletely developed. Their life cycles are about one-thirtieth as long as that of the human. This later point enables us to study the succeeding generations, the heritage of which can easily be controlled. Finally, the anatomical and chemical analysis of the nervous system at different ages can easily be carried on with rats. Thus, in order to determine the causal relationship between diet and nervous function in a strictly scientific manner, our problems must necessarily be investigated first on small animals, of which white rats are the most practicable for our experimentation at this time. We hope to be able to repeat some of our experiments on infants in order to determine directly the applicability of our findings to human beings.

The technic used in depleting the nursing young rats of vitamin B complex merits a special description. It is well known that white rats, unlike guinea pigs, have a prolonged period of infancy. During the first three weeks after birth the young live entirely on the mother's milk. Sure (6) has demonstrated that the quantity of vitamin B in the milk of the nursing mother rat is proportional to the vitamin B content of the mother's diet.

Thus we succeeded in giving the young a diet at first deficient in and later free of vitamin B complex by merely depleting the nursing mothers of yeast and wheat germ which are the main sources of vitamin B complex. The degree of depletion was subject to variation. Some of the mothers have a larger storage of vitamin B complex than others, while the size of the litter varies from one case to another. Even in the same litter, different individuals do not receive the same amount of vitamin B complex from the mother. However, for group comparison, the technic proved to be satisfactory.

Four groups of animals were used in the present investigation. Group I consisted of 60 control animals whose mothers were fed on a normal diet rich in vitamin B complex during the nursing period. Group II, 46 in number, was depleted of vitamin B complex through the mother's diet until they were strong enough to wean. Group III, 40 in number, was raised on a normal diet rich in vitamin B complex during the nursing period although their parents, while nurslings, suffered from the depletion of vitamin B complex. Group IV, 34 rats, was depleted of vitamin B complex during the nursing period in addition to the depletion of their parents during the latter's early life.

The technic of depleting the second generation was the same as that employed in depleting the first generation. Other procedures in feeding, handling and maze training were identical for both generations.

The normal and the vitamin B depleted diets are as follows:

Constituents	Control	B-depleted
Egg albumin (extracted)	20%	20%
Salt-mixture (Osborne and Mendel)	4	4
Dextrin	29	29
Starch	23	35
Agar	2	2
Butter	5	5
Cod liver oil	5	5
Yeast	7	0
Wheat embryo	5	0

Egg albumin, dextrin and starch are all extracted free of vitamin B complex. Preliminary work several years ago led us to include yeast and wheat embryo in the normal diet so that both B(B_1) and G(B_2) are present.

To repeat, our animals were depleted only during the nursing period. After the depleted animals were weaned, they were given a week of vitamin-rich diet so that their body weight was brought nearer to that of the control animals. On the other hand, when the control animals were weaned they were fed on the B-free diet for a few days, just enough to check their

rate of growth without seriously depleting them. From 49 days of age on, each group was placed on exactly the same diet which consisted of Sure's B-free food plus brewer's yeast.

As soon as the animals reached the age of 70 days, they were given a preliminary training in the food box of a standard maze once a day for a week. This was done to inculcate in the white rats the association between maze and food satisfaction, as well as to familiarize them with the general experimental situation. The maze we used contained nine culs-de-sac or blind-alleys and was one of considerable difficulty. It was designed by Professor Harvey Carr of the Psychology Department of the University of Chicago and has been widely used as a standard apparatus in the field of comparative psychology.

After the week of preliminary training, the animals were required to run the maze once daily during the first three days; after this two trials were given a day until the problem was learned. The criterion of learning was 8 correct out of 10 consecutive trials.

The incentive used was a freshly dealcoholized vitamin concentrate[1] from the wheat germ. Sure's B-free food plus brewer's yeast, available with water, was always in the living cage of each animal after the age of 49 days. Thus extreme hunger was avoided. The animals, however, were eager for the incentive used. Prelimi-

[1] Wheatamin.

TABLE I

VITAMIN B COMPLEX AND MAZE LEARNING OF FIRST AND SECOND GENERATIONS

Group	No. of rats	Av. weights during maze learning		Diet while nurslings		Average Maze Scores						
				Generation		Trials			Errors			Time
		Start Maze	Finish Maze	First	Second	Av.	St. D	PE	Av.	St. D	PE	Sec.
I	60	131	181	B Comp. Rich		45	30	2.6	108	66	6.7	1992
II	46	96	173	B " Depl.		106	80	6.9	248	175	17.3	4210
III	40	123	158	B " "	B Comp. Rich	43	21	2.3	122	64	6.7	2093
IV	34	83	161	B " "	B " Depl.	106	86	9.8	245	143	16.5	4120

St. D—Standard deviation. PE—Probable error.

nary work showed that this represented the best procedure in getting the groups to the nearest approximation in their relative strengths of incentive.

The results thus obtained in maze learning are to be compared according to each of the three criteria of measurement; the number of trials before mastery, the number of cul-de-sac errors, and the length of time in seconds required for running the maze.

The comparative average scores of the four groups in maze learning are presented in Table I.

The results may be summarized as follows:

1.—As to the first generation, those animals which had been depleted of vitamin B complex through their mothers' diet during the nursing period are very much inferior in learning ability to the normal animals which received a vitamin rich diet while they were nurslings. The differences are very large and consistent with all the criteria of measurement. Normal rats learn about twice as efficiently as the vitamin B-depleted animals. These data confirm our previous findings with smaller groups.

2.—As to the second generation, namely, the offspring of rats depleted of vitamin B complex in their early life, those animals which were again, like their parents, depleted of vitamin B complex during the nursing period are very much inferior in learning ability to those which, unlike their parents, received a vitamin B-rich diet while they were nurslings. The differences are again very large and consistent with all the criteria of measurement. Vitamin B-rich animals from depleted parents learn about twice as efficiently as vitamin B-depleted animals from depleted parents. These data of the second generation confirm our findings of the first generation.

3.—When the offspring of the depleted animals are brought up on a vitamin B-rich diet, their learning ability is practically equal to that of the normal animals. This seems to suggest that the learning ability of the second generation is not affected by the early depletion of vitamin B complex on the part of the first generation.[2] This may mean that the detrimental effect of vitamin B deficiency upon learning ability is not transmitted to the offspring through the germ plasm. At any rate, the data clearly indicate the possibility of restoring the learning ability of the offspring to a normal level by giving them a normal diet during the nursing period.

[2] There is perhaps a selective factor here as the infant mortality is much higher. See Author's article on Infant Mortality, to be published shortly.

4.—When the offspring of the depleted animals are depleted of vitamin B complex during the nursing period, their learning ability is practically equal to that of the depleted parents. Here again, it suggests that the detrimental effect is not transmitted from the parents to the offspring as the effect is not cumulative through generations. Further work is being done with the third and fourth generations.

5.—The comparison in maze records between the vitamin B-depleted animals and their own offspring brought up on a vitamin B-rich diet proves conclusively that the large differences in their learning ability are not due to the genetic factor of family strain, but to the dietary factor of vitamin B depletion. These data confirm our previous findings obtained by comparing depleted and control litters of the same parentage.

In short, our data prove conclusively that early partial depletion of vitamin B complex is detrimental to learning ability but that the offspring of depleted animals can attain the normal level of learning ability provided they are brought up on a vitamin B-rich diet during the nursing period.

Having established the causal relationship between dietary deficiency and learning ability in experimental animals, we shall consider the applicability of our findings to human beings.

Let us first cite the work of McCollum, Parsons and Kalmbach (7). "In 1919 we sought an opportunity to test on human beings to what extent the principles established in animal experiments apply to the nutrition of children. We sought a group of children who, because of poverty or lack of knowledge on the part of those responsible for their care, were being fed a diet which we suspected to be inadequate for the production of growth and well-being. We discovered an institution in which there were 236 negro children ranging in age from early infancy to twelve years, whose environment and food supply left little to be desired in relation to our objective. Severe malnutrition existed in most of them; it appeared that the cause was probably inadequacy of their diet. An opportunity was offered to improve the quality of their diet in order to observe the extent to which the children would respond to growth and well-being.

"The diet of these children during the period when growth should be proceeding at a rapid rate consisted essentially of cereals, tubers, fleshy roots and muscle meats, and a considerable portion of the food was refined wheat flour in the form of white bread.

"The simplest procedure for improving the quality of this diet without involving additional cookery appeared to be the addition of milk. One group of 42 children received 1 quart of whole milk each per day during

the demonstration period. This was prepared from whole milk powder,[3] by dissolving the proper amount of the whole milk powder in cold water and agitating in a mixer. The other group, designated the check group, was continued on the institutional diet.

"There was a marked change in the behavior of the milk-fed group as contrasted with the check group. The latter were apathetic and very tractable. The discipline of the institution was strict and these children were all obedient. Those in the milk-fed group, on the other hand, soon caused annoyance to their teachers by their restlessness and desires for activity, and were frequently guilty of infractions of the rules."

Next we shall cite Blanton's work (8) in the war zone: While located with the Army of Occupation in Trier, Germany, Blanton had opportunity to study the effects of prolonged under nutrition on the nervous systems and the mentality of 6,500 German school children. Because of the many complaints of the teachers regarding the mental deterioration and the increase in nervous disorders, Blanton undertook a detailed study of the situation. The children were between five and a half and fourteen years of age.

"At least forty per cent of the children in the Volksschulen of Trier, Germany, are suffering from malnutrition to such a degree as to cause a loss of nervous energy. The percentage of children failing to pass their grades has increased from an average of eight per cent in pre-war years to fifteen per cent in 1917 and 1918. It is estimated about half of this seven per cent increase in retardation has been due to malnutrition, the other half has been due to war conditions. There has been a lowering of the whole standard of school work caused chiefly by malnutrition but partly by war conditions in general; half of the children who in pre-war time did superior work now do average work, and the percentage of children who did inferior work has been increased from twenty to more than thirty per cent. The specific changes noted in the children, caused by malnutrition, are: (a) lack of nervous and physical energy, (b) inattention during school hours, (c) poor and slow comprehension for school tasks, (d) poor memory for school work, and (e) a general nervous restlessness while in school."

Finally, we shall cite the work of Hoefer and Hardy (9). These investigators reported the results of tests and measurements made on 383 children, ranging in ages from 7 to 13 years and grouped according to the length of the period of exclusive breast feeding.

"Mental development was measured by individual psychologic examinations with the Stanford Revision of the Binet-Simon intelligence

[3] Klim.

test and the Pintner-Patterson performance scale (a nonverbal intelligence test), and by a group educational test, the Stanford achievement test. The results for these tests are recorded in terms of quotients which express the relationship between the child's ability and what is expected of his age.

"From an analysis of the results on mental development, it will be seen that the artificially fed were inferior in all standardized measurements to those breast fed from four to nine months, and, with one exception, to those breast fed three months or less. They, however, equaled or excelled those breast fed from ten to twenty months. In learning to talk, the artificially fed were the most retarded of all the groups. The importance of breast feeding is further emphasized by the fact that among those children having the highest intelligence quotients, not a child artificially fed rated as high as 130, while 5 per cent of those breast fed were above this score."

In the light of our experimental data, these investigations on children, although suggestive in character, seem to indicate that the relationship between dietary deficiency and higher nervous function also exists in human beings.

The next problem was raised by Barnett Sure (10) as follows: "Our illuminating findings that nursing young of the albino rat need approximately 100 times as much of vitamin B (calculated in terms of dehydrated yeast) as vitamins A and D (as furnished by a brand of cod liver oil employed by us since 1921) for growth during the nursing period brings up the question: Do infants, breast or artificially fed, receive adequate amounts of vitamin B?"

"The usual dietary of an infant in the United States, up to its third month, consists of human milk or cow's milk, to which has been added a sugar or a syrup and possibly a milled cereal, with some orange juice and cod liver oil. When this diet is analyzed for its vitamin content, A, C and D are found to be present, but vitamin B is represented only in milk and in orange juice, and in these only in limited amounts. From the foregoing discussion it is plain that only minimal, and in some circumstances subminimal, amounts of vitamin B are to be found in such a dietary (11).

In view of the widespread chronic partial deficiency of vitamin B complex in Asia, Europe and America, not to mention Australia and Africa, due to the large consumption of polished rice and highly milled white flour bread, it is an urgent necessity that vitamin B complex should be supplemented in sufficient quantity to the diet of the expectant and

nursing mothers as well as to that of the breast and artificially fed infants and older children.

To summarize, our data on experimental animals have proved conclusively that early partial depletion of vitamin B complex is detrimental to higher nervous function as measured by maze learning ability; but that the offspring of the depleted animals, *i.e.*, those that survive the high infant mortality, can attain the normal level of learning ability provided they are brought up on a diet rich in vitamin B complex.

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THE EFFECTS OF RADIANT ENERGY ON MILK ANEMIA IN RATS*

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FROM a review of the literature it may be concluded that normal men and animals on an ample, balanced diet are able to live under conditions in which sunlight or artificial radiation is entirely absent or much reduced for long periods of time, perhaps indefinitely, with no resulting anemia. Conversely, increasing the amount of radiation which a man or animal receives, up to a limit that does not produce harm, does not produce changes in the blood picture other than to alter the blood volume temporarily. On the other hand, in the diseased organism there is considerable evidence that radiation often results in improvement of the blood picture. The older experimental work on this subject is unsatisfactory and contradictory, see the reviews by Reed (1925), Hardy (1927) and Laurens (1928). There was no standardization of the source of radiation; in fact, no appreciation of what the source was really emitting. Such work naturally cannot be repeated.

Osato and Tanaka (1929) report that quartz mercury arc radiation mobilizes the iron reserve in the spleen and liver and hastens hemoglobin regeneration in dogs, rabbits and rats made anemic by venesection. Laurens and Mayerson (1931) have just shown that, although carbon and mercury arc irradiation results in a marked and persistent increase in the number of erythrocytes and reticulocytes in hemorrhagic anemia, such treatment fails to accelerate the regeneration of hemoglobin. In acetylphenylhydrazine (pyrodin) anemia, on the other hand, Mayerson and Laurens (1931) found that such radiant energy, although it had no effect on the downward course of the anemia, hastened the regeneration of hemoglobin as well as that of the red cells.

The effects of radiation on a nutritional type of anemia have received

* The data in this paper are taken from a thesis submitted to the Faculty of the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1930. The investigation was carried out under the direction of Professor Henry Laurens.

Preliminary reports on the results have appeared in the *Proceedings of the Society for Experimental Biology and Medicine*, 1929, xxvi, 251; 1930, xxvii, 892.

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little experimental study. Mathews, Doyle and Whiting (1929) irradiated pigs with the quartz mercury lamp and with sunshine in an attempt to benefit a naturally occurring anemia. Little benefit as regards the anemia was noted in the pigs irradiated with the quartz lamp although the mortality of this group was lower than in the non-irradiated pigs. Young growing pigs kept in the sunshine showed at the end of 38 days hemoglobin levels which were higher than in control animals. Hart and co-workers (1927) noted no beneficial effects of mercury arc radiation on the blood of rabbits rendered anemic by a milk diet. These observations were incidental to another problem and apparently not based on a large series of results. It is reasonable to inquire whether or not radiant energy would be effective in promoting the metabolism of iron when that substance is low in the diet. Accordingly, it was decided to produce anemia in rats by feeding them an iron-poor diet (milk) and to observe the effect of radiation in preventing or curing this anemia.

The experimental procedure was simple. Rats from a healthy albino strain bred in our own laboratory, were divided into groups of about 10 each, when 21 to 28 days old, care being taken to keep litter and sex distribution equal. Some of the groups were exposed to varying amounts of radiant energy; others receiving no irradiation served as one group of controls. Other groups of controls consisted of rats on the stock diet receiving no radiation and of rats on the stock diet receiving radiation. The observations were continued for from 10 to 14 weeks. The stock diet was a commercial meal (Maynard, 1930) supplemented by lettuce twice a week. Powdered whole milk[1] was used in preparing the milk diet. By purchasing large quantities of the powder at a time from the same lot, a uniform diet was insured over a long period of time, an advantage which we could not obtain by the use of our available source of dairy milk. Solutions containing 12.5 per cent in distilled water were fed *ad libitum*. Such solutions, according to the manufacturers, have approximately the same composition as fresh dairy milk. The drinking water supplied was distilled. Care was taken to prevent the transference of food material or excreta from one cage to another.

Hemoglobin determinations were made once each week on blood from the tail with a Newcomer instrument standardized by oxygen capacity determinations with the Van Slyke manometric apparatus. The rats were weighed weekly. Red and white counts and red cell volume readings (hematocrit) were made at the end of, and once or twice during, each

[1] Klim.

experiment. Neubauer chambers were used for the cell counts and Van Allen tubes for the hematocrits. From the values obtained for the hemoglobin, red cell counts and volume, the average corpuscular volume and saturation were calculated. The use of such figures permits the expression of the data relating to the red cell in absolute values rather than in terms of "indices" or "coefficients."

The sources of radiant energy used were a "Pan-Ray-Arc"[2] burning National Therapeutic A "Sunshine" carbons and an air cooled Cooper-Hewitt quartz mercury vapor lamp. The carbon arc as operated emits an average of 0.820 gm.-cal. per sq cm. per min. of energy incident at 1 meter and has a distribution of 5 to 6 per cent ultra violet, 29 to 31 per cent luminous and 63 to 66 per cent infra red. The mercury lamp emitted 0.0619 gm.-cal. per sq. cm. per min. incident at 1 meter distributed as follows: 13 per cent ultra violet, 7 per cent luminous (400–1400 $m\mu$), and 80 per cent infra red. Further details are given in the papers by Laurens and Mayerson (1929 and 1931).

The hemoglobin of the rats at birth was about 14 gms. per cent. At 3 weeks of age, when they were weaned, it had dropped to 8 or 9 gms. per cent. When rats are placed on a milk diet the hemoglobin continues to decline, dropping to about 5 gms. per cent in from 4 to 6 weeks and many of the animals die. In those that survive the hemoglobin increases to about 6 gms. per cent and the rats live on with their hemoglobin at this value. When placed on the standard calf meal diet at weaning, the hemoglobin immediately begins to rise and reaches about 15 gms. per cent at the end of 10 weeks. Growth on the milk diet was not, as a rule, greatly retarded during the first 2 to 4 weeks, but by the end of 10 to 12 weeks such rats weighed only 140 to 160 grams as compared with 190 to 240 grams for rats reared on the calf meal. When first placed on the milk diet rats almost universally develop a severe diarrhea, large quantities of mucus being sometimes observed in the feces As above stated the death rate is high. Gross examination fails to reveal intestinal lesions. The diarrhea disappears at about the same time that the slight, spontaneous rise in hemoglobin takes place. Perhaps the intestinal disturbance is a factor in the genesis of the anemia, preventing adequate absorption of the small amounts of blood-building material in the diet. The fall in hemoglobin is most rapid during the first 4 to 6 weeks on the milk diet which is the period of most rapid growth. During the following weeks growth is considerably slower and consequently there is a balance struck between

[2] Made by Atlas Electric Devices Co.

the requirements for and the intake of hemoglobin-building substance. Rats on the milk diet and growing abnormally slowly, maintained higher hemoglobin concentrations than did rats growing rapidly. None of the rats showed evidences of rickets.

At the end of some of the observational periods the female rats were placed on the stock diet and subsequently mated with males that had been reared on this. In this way many litters were obtained and designated as "second-generation rats." These when weaned and placed on the milk diet, became anemic in a slightly shorter time and showed less tendency to recover spontaneously than did rats from the stock colony, although the lowest levels reached were about the same in both groups. Similarly "second-generation" rats were placed on the stock diet and mated with healthy males giving litters which were designated as "third-generation rats." Beyond the third generation the breeding propensities became so poor that it was not possible to continue in this manner.

The red cells of the anemic rats showed great variation in size and shape, both of which features are characteristic of secondary anemia and may be taken as one index of its severity. The cells were much below the average in size (see Table I), the size tending to vary inversely with the severity of the anemia. About 15 per cent of the cells in the anemic bloods

THE EFFECT OF RADIANT ENERGY ON MILK ANEMIA, EXPERIMENT 16. AVERAGES OF GROUPS OF ABOUT 10 EACH VALUES OBTAINED IN THE 10TH WEEK.

Conditions	Weight gms	Hb level gms per cent	Hematocrit per cent	R.B.C. millions	Corp. vol. cu. μ	Corp. Hb gms. $\times 10^{-12}$	Saturation per cent
1. Stock diet No irradiation	216	16 3	42 0	8 23	51 0	19 8	38.8
2. Milk diet No irradiation	140	6 1	19 5	6.68	29 2	9 1	31.3
3. Milk diet, Hg arc 5 min. daily	160	11 3	34 5	7.70	44 8	14 7	32.8
4. Milk diet, Hg arc 15 min. semi-weekly	163	13.0	35 0	7.84	44 6	16 6	37.1
5. Milk diet, Hg arc 15 min. daily	168	12 4	33 0	8.52	38.8	14 6	37.6

were polychromatophylic. The hemoglobin content of the cells was low and distributed about the periphery of the cells leaving the central portion clear. The red cell counts were not much lower in the anemic than in the normal rats. The resistance of the red cells to hypotonic solutions was

markedly increased Total white cell counts in the anemic animals were lower than in the normal ones, this being due chiefly to diminution in lymphocytes, the neutrophiles being unchanged or increased.

The hemoglobin values in 11 of 18 groups of rats given various amounts of quartz mercury arc radiation were definitely higher than in the controls, slightly higher in 4, the same in 1, and lower in 2 groups than in the controls. The periods of irradiation varied from 4 minutes to 25 minutes daily and from 15 minutes to 35 minutes semi-weekly. In some experiments the irradiation was begun when the rats were first placed on milk, in others not until after the hemoglobin had dropped to low levels. The results of some of the best experiments are given in Figure 1. In general, the radiant energy failed to prevent the initial fall in hemoglobin al-

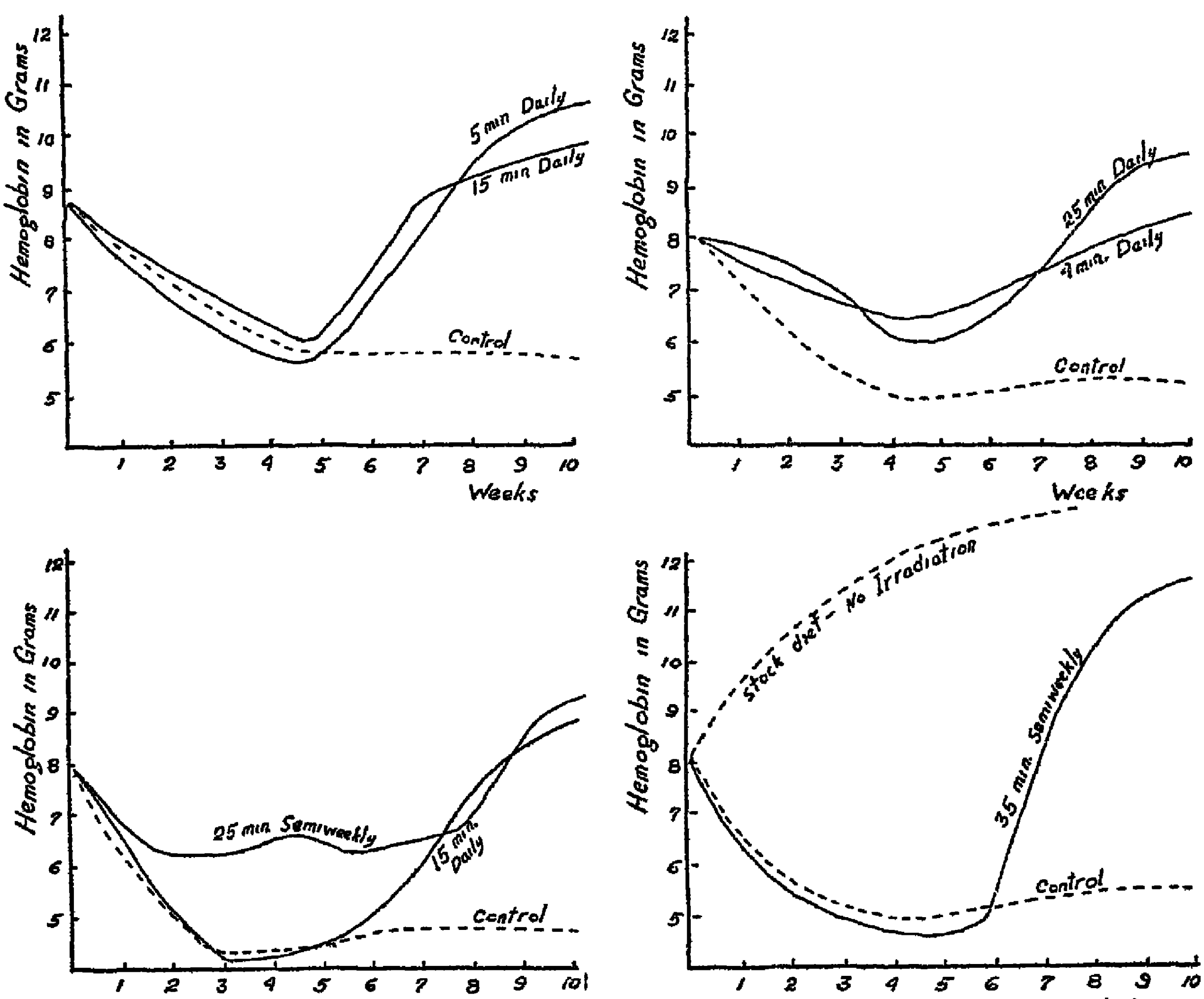

FIG. 1. Graphs showing the effect of mercury arc radiation on the hemoglobin content of rats maintained on a milk diet Each curve represents the averages of a group of 7 to 10 individuals. In each of these experiments the radiation was given throughout the 10 weeks' period.

though in several series this fall was retarded and diminished. It was after the fourth week, when the hemoglobin had reached its lowest level, that the effect was greatest. Not only was an increase in the hemoglobin con-

tent of the blood noticed, but the red cells became more normal in their number, saturation, size, shape and staining. The results of a typical experiment are shown in the table (p. 520). Small doses (4 to 5 minutes daily) were in general as effective as larger doses (15 to 25 minutes daily). Growth was usually improved by small or moderate doses, but there was a tendency for larger doses to inhibit it. The mortality in general was lower in the irradiated than in the non-irradiated groups. The effect on the white cells was inconstant.

The differences in hemoglobin between the irradiated and non-irradiated groups were not large but the weighted mean value for all animals surviving 10 weeks or longer is 8.29 ± 0.130 gms. in irradiated animals, and 5.80 ± 0.096 gms. in the controls (Fig. 2). The difference, as small as it is, often meant survival instead of death in the very anemic animals. In

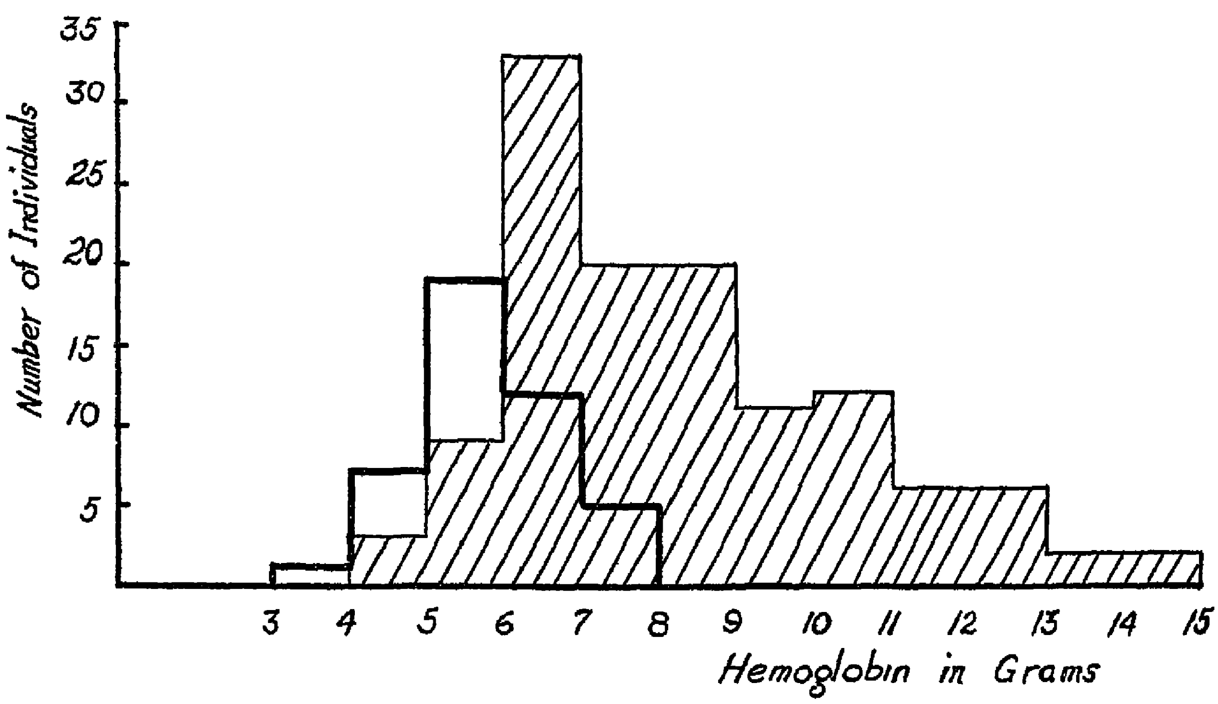

FIG. 2. Frequency diagram showing the effect of 8 weeks' irradiation with the mercury arc on hemoglobin content. The area under the heavy lines is for non-irradiated, the shaded area for irradiated animals

some of the experiments where the irradiated animals had 11 to 13 gms per cent of hemoglobin as compared with less than 6 gms. per cent in their controls, there can be no question of the physiological importance of such an increase. Here the sleek, well nourished, active irradiated rats stood in marked contrast to the scrawny, emaciated, inactive controls.

Irradiation with the flaming carbon arc resulted negatively. Of 15 groups, the hemoglobin values in 8 were equal to or lower than their controls, in 7 slightly higher, but in only one group sufficiently so to be indicative, 7.9 gms. as compared with 5.7 gms. in the control. The energy

from the carbon arc was given in a wide variety of doses, varying from 5 minutes to an hour daily and 5 minutes to an hour semi-weekly at a distance of 1 meter. The reason for the effectiveness of the mercury lamp as contrasted with the ineffectiveness of the carbon lamp is not apparent, although it seems clear that the difference between the activity of the two sources was not dependent upon the total amount of energy. It would be unwarranted to conclude that the carbon arc has no beneficial results on the blood picture, but certainly under the conditions of our experiments no such effects were noted.

A group of 16 rats exposed to sunshine for a half hour daily, beginning at 10 o'clock, through June and July, had hemoglobin values at the end of 7 weeks slightly higher than in 9 control rats exposed only to the subdued daylight of the stock room, 8.4 gms. as compared with 6 7 gms.

On a diet entirely devoid of hemoglobin-building substances, radiation could hardly produce favorable results. The rats in our experiments were undoubtedly getting small amounts of iron and other minerals in their diet, enough to maintain a balance at low hemoglobin levels over a long period of time The function of radiation may have been to improve the economy with which this small intake was utilized so that a balance at a somewhat higher level could be maintained. In a few experiments iron was added to the diet, but hemoglobin regeneration was no more rapid in the irradiated than in the control groups. The quantity of iron used (1 mgm. per rat daily) was probably large enough to produce regeneration at its maximum rate so that it could not be stimulated further by any other means.

The question may arise as to what part blood volume changes play in explaining the observed results. No direct measurements of the blood volume were made. Mayerson and Laurens (1928) observed that irradiation with the carbon arc resulted in an increase of 6 to 37 per cent in the plasma volume of dogs, but that recovery to normal volume took place within 5 hours. Most of our observations were made 24 hours after the last irradiation. The fact that an increase in the size and saturation of the red cells was observed concomitantly with the increase in hemoglobin argues against the changes being due to blood concentration. Further, it is hardly conceivable that blood volume changes could bring the hemoglobin values from 5 or 6 grams to 12 or 13 grams as in some of our experiments. Moreover, the hemoglobin values were practically the same whether the determinations were made one day or several days after the last period of irradiation. The results are also not explained on the basis of inhibition of growth since some of the animals which showed the best

hematopoietic response also showed marked acceleration in the rate of growth, and, contrariwise, some whose growth was inhibited, failed to show increased hemoglobin concentration.

In all probability the radiation, when effective, stimulated body activity, bringing about improvement in the general nutritional state, and this is reflected in part in the improvement in the blood picture.

Summary

Irradiation with a quartz mercury arc has a slight but definite effect in increasing the hemoglobin content of the blood of rats rendered anemic by a milk diet. The number, size, and saturation of the red cells are also increased A flaming carbon arc, from "Sunshine" carbons, had no effect.

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Editorial Review

SOME ESSENTIALS OF A GOOD NUTRITION EXPERIMENT

A PERFECT experiment in any field of science may be said to be one that has been planned and conducted in such a way that the results obtained are susceptible of only one interpretation. Only such an experiment can amount to a demonstration. To the extent that this ideal has not been realized, in that more than one interpretation of the results is possible, to that extent the experiment has established only a certain limited probability in favor of the conclusion drawn. But this requirement of a perfect experiment is difficult to realize, particularly in the biological sciences. Two illustrations, one in a physical science and one in the science of nutrition, may serve to emphasize the difficulties.

In 1887 the famous Michelson-Morley experiments were undertaken to measure the velocity of the earth through the ether. The general principle upon which these experiments were based was that the velocity of light, as observed on the earth, would depend upon the direction of the light relative to the direction of the earth's motion through the ether, the extent of this dependence affording a measure of the earth's velocity. The technic of the experiments need not be described here except to point out that the apparatus used was so sensitive that a velocity of the earth as slow as 1 meter per second could be detected. The experiments were repeated a number of times but always with the same result, namely, that the velocity of light was quite independent of its direction. Hence, the earth appeared to be stationary in the ether. Here is an experiment, performed with the utmost precision, which seemed susceptible of only one interpretation. However, the interpretation is quite inconsistent with the known fact that the earth is traveling around the sun at a speed of nearly twenty miles a second. In such a situation, there must be an alternative explanation of the experimental results. An alternative explanation was later presented. It was shown, on the basis of the electrodynamical theory then accepted, that bodies moving through ether are contracted in the direction of their motion, and that in the Michelson-Morley experiments the differential contraction of the apparatus, and in particular the contraction of the measured paths over which the light traveled, may have been just sufficient to compensate for the differences in the velocity of light induced by differences in its direction. However, other methods of measuring the effect of direction upon the velocity of light have also given negative results, al-

though these methods have not involved the use of material measuring rods, so that the alternative explanation of the Michelson-Morley experiments does not appear to embody the whole truth. Hence, what appeared to be a precise experiment, capable of giving results susceptible of only one interpretation, seems, in fact, quite uninterpretable at the present time. In the words of Sir James Jeans: "It appeared then that if the earth moved through the ether this motion was concealed by a universal shrinkage of matter, and this shrinkage was in turn concealed by some other agency or agencies whose wit, so far, appeared to be greater than that of man."

In the physical sciences experimental conditions may be much more rigorously controlled than in the biological sciences. Furthermore, the problems in the physical sciences are so much more often open to direct attack. It is to be expected, therefore, that a perfect experiment would be more difficult to realize in the biological sciences than in the physical sciences. Let us consider a well-controlled nutrition experiment concerned, let us say, with the question as to whether or not a certain amino acid is indispensable for growth. A ration is prepared including a low percentage of a protein known to contain only traces of the amino acid in question, but including in adequate concentrations all other essential growth factors, in so far as current knowledge of such factors extends Another ration is prepared, similar to the first except that a small percentage of the amino acid is substituted for an equal percentage of starch. Two groups of comparable experimental animals are now selected, one group to receive the first ration and the other the second. To make the experiment more directly and easily interpretable in terms of the relative growth-promoting values of the two rations, the animals are fed individually and the amounts consumed are regulated in each case so that the rates of gain are the same, within narrow limits, for all animals. After a feeding period of several weeks duration, the total intake of food is computed for each animal. It is found that less food was required by all of the animals on the second (supplemented) ration than by any of the animals on the control ration to produce the same increase in body weight The conclusion seems clear that the amino acid included in the second ration was indispensable for growth.

However, there are other possible interpretations of this experiment, no one of which can be ruled out on the basis of the data obtained:

1. The gains in weight in the two groups of animals may differ in composition. If the gains induced by the supplemented ration contained more water or more fat or both than the gains induced by the control ration, the former ration may be no more efficient than the latter in the promotion of growth, or more specifically, in the promotion of protein synthesis.

2. The basal metabolism of the experimental animals may have been depressed by the added amino acid, in which case less food would be required to produce a given gain in weight because a greater proportion of the food eaten would be available for this purpose.

3. The activity of the animals may have been depressed by the added amino acid, again leaving more food available for growth The effect of different rations upon the voluntary activity of experimental animals is almost a virgin field of investigation.

4. The added amino acid may be serving as a precursor for some indispensable dietary constituent (vitamin) not at present known or characterized and not contained in adequate amount in the basal ration. Its function in this case may be quite unassociated with protein synthesis.

It may be objected to this analysis of the experiment that the alternative explanations are quite improbable, even though not inconceivable. The objection cannot be over-ruled, but it may be pointed out that any assessment of the probability of an event is based only upon current knowledge, which may be seriously incomplete. The progress of science inevitably changes the probability of the occurrence of events. What seems improbable today becomes quite probable tomorrow, or it may even be established with certainty. I think it must be admitted that the experiment under discussion does not *demonstrate* anything in any strict sense of the term simply because its results are susceptible to a number of possible interpretations, although the most obvious interpretation *seems* to be far more probable than the others.

The same analysis could be made of any experiment in which the relation between dietary constituents and the rate of increase in body weight is being established This is true either because the composition of gains in weight depends in part upon the composition of the diet consumed, or because the food available for increase in weight is the difference between the total food consumed, which may be controlled, and the food used for the maintenance of life and for voluntary activities, which can be only incompletely controlled by the investigator.

The situation discussed above seems typical of experimentation in nutrition in so far as it relates to the difficulties of interpretation. It impels caution in the formulation of conclusions on the one hand, and on the other hand it stresses the importance of a precise technic and of a rigorous control of experimental conditions, since the most rigorous control will not remove all of the uncertainties of interpretation. Over-confidence in the most obvious or the most probable interpretation of an experiment and a corresponding over-positiveness in its formulation may perpetuate a mis-

conception and seriously impede scientific progress, while excessive caution will at most delay temporarily the establishment of a truth

While it may not be possible with many types of nutrition problems to demonstrate anything in the strictest sense of the term in a single experiment, because of a multiplicity of possible interpretations, it is sound policy to reduce the number of possible interpretations to a minimum. This can be done only by extending control in so far as feasible to every experimental condition that could possibly influence the animal function under observation and measurement, so that control and test animals are subjected in an equal degree to all experimental conditions except the one being studied, or that, in control and test periods, experimental conditions are similarly equalized. It is, of course, impossible to consider in this paper all of the implications of this proposition. Attention will be confined, therefore, to only a few of them.

It is generally recognized that the problems of nutrition are essentially statistical in nature in the sense that they require an accumulation of data for their solution. Experimental animals, no matter how carefully they have been selected, exhibit variability in functioning under the same conditions of treatment. Hence, experiments involving only one or two or three animals can only rarely be expected to furnish a decisive answer to an experimental inquiry. It is a common practice to include a larger number of animals on each experimental treatment, though unfortunately there is no common agreement either on the precise number to use or on the logical method to use in determining in each case how many may be necessary. Statistical methods can aid in avoiding, on the one hand, the embarrassment attending the conclusion of an experiment that has yielded such a small amount of variable data that no definite interpretation is possible, and, on the other hand, the quite unnecessary labor and expense in obtaining far more data than are required to clinch the point at issue. But unfortunately statistical methods are but slowly coming into vogue in nutrition laboratories.

After deciding, arbitrarily or logically, how many animals are to be employed, one must next decide whether to feed them in groups or individually. Both methods are quite commonly followed. It seems well, therefore, to consider which is the best. Group feeding is the more economical of labor. This is perhaps its greatest if not its only advantage over the individual feeding method. If it possessed no disadvantages, the saving of labor, or the ability to carry with the same amount of labor a larger number of experimental animals would be decisive, but if the saving of labor can be secured only by a serious sacrifice in precision, because of a less complete

control of the experiment, then it should not be accorded any considerable importance.

Another advantage of the group-feeding method that is urged particularly in livestock experimentation or in experimentation concerned more or less directly with the practical problems of livestock farming, is that it is a more natural method of feeding, involving no restriction on appetite except that imposed by the competition of animals within the group. However, natural conditions mean uncontrolled conditions and the prevalence of uncontrolled conditions means ineffective experimentation. If science were compelled to forego entirely the control of conditions in its seeking for truth, and to make its observations only under those conditions of living "natural" to the species of animals being observed, it would have to dispense entirely with the experimental method. While nobody would perhaps take this extreme position, there are apparently many who hesitate at each step in the imposition of experimental control for fear that the further one proceeds from the "natural," the less applicable the observations secured become to animals in their "natural" environment. This attitude leads to a compromise somewhere in the planning of the experiment between the demands of good experimentation and the fear of the effects of limiting the "natural," or the "instinctive," behavior of experimental animals.

However, it seems a fair judgment that this fear is a baseless one unless there is some objective evidence that a given experimental procedure will yield spurious results of no general applicability, or unless there is some good logic leading to the belief that such would be the case. In attacking the overwhelming majority of problems in the science of nutrition it seems a greater evil to compromise with the demands of good experimentation than to risk the possibility, generally remote, that some of these demands may limit the usefulness of the evidence obtained. In the former case all too frequently the evidence obtained is too vague for any sure application whatsoever; in the latter case, at the very least, the stage has been set for the production of a definite contribution to knowledge.

The disadvantages of the group-feeding method are serious. In applying this method, the investigator removes the possibility of measuring the food intakes of individual animals and also of controlling them. If, as in livestock experimentation, he is concerned with measuring the amount of food required to produce a unit of gain in body weight, he has deprived himself of all means of measuring the experimental error of the average value of this ratio. An average entirely dissociated from the individual items of which it is composed is a weapon of dubious value in attacking

scientific problems. It may be as good as its face value (or nearly as good), or its effectiveness may be seriously impaired by a large experimental error. Whether the former is true or the latter can be decided only by consulting the individual items, whose number and variability will determine the experimental error of the mean result. The difference in significance between a group of widely variable data and another group possessing the same mean value but a much smaller variability is analogous to the difference in climate between two localities possessing equal mean annual temperatures of 70°F., but mean ranges in one case from −20°F. to 105°F., and in the other case from 35°F. to 85°F. To say that the climates are the same because the mean annual temperatures are the same is seriously to distort the truth.

Any measurement made on the individual animals of a group, be it rate of gain in weight, blood composition, bone composition, or the like, will presumably be affected by the amount of food consumed unless definite evidence to the contrary is at hand. The assumption, for example, that the extent of calcification of the bones is not affected by a variable consumption of a ration possessing a constant content of vitamin D and a constant calcium-to-phosphorus ratio, is indefensible until it has been established by direct experiment. The variability in these measurements within experimental groups fed together may be due to a considerable extent to a variable intake of food. But whether this is true and to what extent it is true cannot be told with no records of individual consumption available. The variability in the measurement cannot be interpreted with reference to the intakes of food, it cannot be corrected statistically to a constant intake of food, nor can it be actually reduced by an equating of individual food intakes. Hence, the experimental errors of average measurements obtained from animals fed in groups are needlessly large

The advantage of individual feeding is the possibility it presents of observing and controlling the behavior of each unit in a nutrition experiment While there are advantages in merely observing this behavior, the greater the extent to which it is controlled the more nearly will the experiment yield results that are precise and susceptible of clear-cut interpretation.

The control of the consumption of food by experimental animals is of great importance in the production of a good nutrition experiment, unless, of course, the experiment is one designed to test an effect on appetite Among animals on the same ration, the equalization of food intakes will reduce the experimental error of the average results for the group. Between two groups of animals subsisting on rations whose nutritive effects are being measured, an equalization of food consumption will establish a definite

relation between the one difference in composition of the rations and any statistically significant difference observed in animal functioning. Without such an equalization of food consumption the difference observed in animal functioning cannot be definitely related to the one difference in composition of the rations, because a difference in the amount of food eaten might alone affect animal function.

Since *ad libitum* feeding of experimental animals is so extensively practiced in nutrition laboratories, the bearing of this practice upon the interpretation of experimental results may well be considered here. Let us take as the simplest case, two experimental animals, one being fed one diet, which we will call the control diet, and the other being fed a second diet differing from the first in one particular only, which we will call the test diet. If these two animals are fed *ad libitum*, a difference in food intake during a feeding period of several weeks will always occur. We will assume that the animal on the test diet consumes the greater amount of food, and that it gains faster, or recuperates faster from a nutritional anemia, or deposits mineral in its skeletal tissue faster, or exhibits a more favorable functioning in some other respect, depending upon the purpose of the experiment. This more favorable functioning on the test diet could be referred directly to the difference in composition of the two diets were it not for another difference in experimental conditions, namely, the difference in the consumption of food. Of the possible causes for this difference in the consumption of food, we may consider the following:

1. It is the result of purely fortuitous causes, entirely unrelated to the different compositions of the diets: an expression of the "individuality" of the animals
2. It is the result of a difference in the composition of the diets, either
   a. Unrelated to their inherent nutritive values, but in some way causing a differential stimulation of the appetite, or
   b. Related directly to their inherent nutritive values. In this case the test ration has been consumed in the greater amount because it is better balanced and better able to promote animal functioning, although in this case, as in *a*, the physiological mechanisms involved are not understood.

In each of these cases it would seem advisable to remove the effect of the difference in the consumption of food to permit the establishment of a direct relation between the difference in composition of the diets and the observed difference in animal functioning. In the first case, the difference in food intake is of the nature of an experimental error, being the result of uncontrolled factors. Its elimination could work only to the advantage of

the experiment. In the second case, under *a*, the difference in food consumption has been induced by dietary factors not concerned in the promotion of animal functioning. The observed difference in animal functioning may have been produced simply by the greater consumption of the test diet, or if the test diet is the better balanced, it is an exaggerated expression of this better balance. In either case an equating of the food intakes of the animals would have been advantageous in removing a confusing influence, either leading the unwary investigator to a false interpretation, or exaggerating the effect it is desired to measure.

In the last case, the better nutritive balance of the test ration has really produced two effects, namely, it has induced a greater consumption of food, and also, apart from the first, it has induced a more favorable physiological response. If the purpose of the experiment were to measure the difference in physiological response induced by these two diets, it would seem that this purpose would be better served by removing the former effect. The difference in physiological response would not be obscured by this control procedure. On the other hand, if it were desired to measure all nutritive differences of the two rations, the wiser procedure would seem to be to measure each separately. For the most effective progress, scientific problems should be factored into their ultimate terms.

In evaluating the importance of the control of the food consumption by experimental animals, which means limiting the intake of food of some of the animals at least below the amounts that they would voluntarily consume, it should be realized that most experiments in nutrition are concerned, not with obtaining and measuring the maximum nutritive effects of rations, but with measuring their comparative effects. While maximum effects can be obtained only under unrestricted conditions, comparative effects can be measured accurately only under the restricted conditions of a well-controlled experiment.

The interpretation of experimental data, as well as the control of experimental conditions, is not a simple problem nor one in the solution of which universal agreement can be expected. Surely the first step should be to enumerate all the possible interpretations. It will often be found that more than one interpretation is possible, but, if the experiment has been carefully planned and carried through, it should be found, either on the basis of the data obtained or on the basis of other information, that of all the possible interpretations one is considerably more probable than any of the others. The formulation of this outstanding interpretation into a definite conclusion doing full justice to the data without over-emphasizing their importance or significance is preeminently a judicial task.

The formulation of a positive conclusion from an experiment in which, for example, an effect of a given nutritive factor on animal functioning has been established, seems to be in a different category from the formulation of a negative conclusion from an experiment in which no such effect has been observed In the former case, if the effect has been secured by the use of good technical methods and if it has been established on the basis of evidence that is statistically sound and adequate, it may be stated with considerable assurance that a causal relation has been established. For example, it may be stated definitely, if the above conditons are satisfied, that copper will stimulate hemoglobin production, or that carotin will induce recovery from the effects of vitamin A deficiency, or that certain nitrogenous extractives of meat will induce kidney damage. From the results of more exacting experiments it may be justifiable to conclude that the irradiation of ergosterol induces the production of vitamin D, or that the vitamin B complex consists of at least three constituents. These are positive conclusions which may be stated as such because any possible alternative explanations of the experimental data have been disposed of in a series of auxilliary experiments.

But what conclusion is justified when no effects are observed as the result of the imposition of a certain experimental condition? The choice lies between two conclusions: either the non-committal statement that no evidence has been obtained that the given condition is capable of producing an effect, or the outright statement that the given condition is incapable of producing an effect Let us illustrate the situation. An investigation has been undertaken to find out whether the creatine content of muscle is affected by the amount of protein consumed. The significance of the question lies in the fact that arginine, one of the amino acids found in protein, is a probable precursor of the muscle extractive creatine. Two groups of rats are fed two different diets, one high in protein and one low in protein, and after 2 or 3 weeks all rats are sacrificed and selected muscles analyzed for creatine. No statistically significant difference is found between the creatine contents of the muscles of the two groups of rats. What conclusion should be drawn? Should it be said simply that no evidence has been obtained in favor of the view that the creatine content of muscles may be increased by high protein feeding, or should it be said that high protein feeding is incapable of increasing the creatine content of muscle? The latter statement can be shown to be untenable. In the first place it fails to consider that the experimental error, represented by the variations in creatine content among rats on the same diet, may have obscured a small effect of the high protein diet. Its greatest weakness, however, is in assuming that

under other conditions also no effect of protein feeding will ever be observed. It implies with no justification that negative results would also be obtained with rats of different age, with feeding periods of different duration, with different kinds of dietary protein, etc. A fair judgment would be, therefore, that if the conclusion from this negative experiment is thrown into a positive form, i.e., the assertion that high protein feeding is incapable of increasing the creatine content of muscle, the modifying clause "under the conditions of this experiment" should be appended.

Negative experimental evidence thus may be the result of any one of three possible circumstances:

1. The condition imposed has no causal relation with the thing measured, so that variation in the condition cannot exert any effect on the measurements taken.

2. The condition imposed has a small effect upon the measurements taken, but this effect is entirely obscured by the experimental error prevailing, either due to technical methods that are not sufficiently accurate or to an amount of data that is not statistically adequate.

3. The condition imposed has a considerable effect on the measurements taken, but the selection of other experimental conditions was unfortunate for the demonstration of a causal relation, because inadvertently some condition has been imposed under which the relation does not hold

To establish the first interpretation the other two must be shown to be inapplicable. The removal of the second interpretation would seem to be purely a matter of technic, but actually it is impossible to say at any time that small or even infinitesimal effects have not been obscured by the variability in data secured under like conditions, or that small conversions of one substance into another in metabolism have not been missed. If such small effects are of no significance to the problem, then the second interpretation will not complicate matters.

In some problems, however, small effects are rather to be expected, and the failure to detect them gives no assurance that they do not exist. Until about 20 years ago chemical examination of the blood of animals made during the course of protein digestion failed to reveal any increase in the amino acid content, although it was known that amino acids were being liberated in the intestine. This purely negative evidence was unfortunately given a positive significance by concluding that amino acids do not gain access to the blood from the intestine. In explaining this imagined situation it was assumed that the amino acids resulting from protein digestion were resynthesized into protein in the intestinal walls during absorption,

and that these proteins were then taken up by the blood for the nourishment of the cells. Although no direct evidence was ever obtained in support of this theory, it gained wide currency because of the scientific prestige of its advocates, and it was not abandoned even when the only excuse for its inception was removed by the definite proof, by the use of better chemical methods, that amino acids are in fact absorbed as such from the intestinal tract.

Again, about sixteen years ago, two water-soluble vitamins were recognized, one the vitamin that prevents polyneuritis in animals and beri-beri in man, the other a growth-promoting vitamin. But at that time some rather perfunctory studies were made of the chemical properties of these vitamins and of their distribution in foods. The experimental methods employed were inadequate to afford a sharp distinction between the two vitamins in these respects and upon this purely negative evidence the belief was founded that the two vitamins were identical. For more than ten years this conclusion was accepted with very little question. But as the effectiveness of experimental methods increased data contradictory of this conclusion accumulated and more and more doubt was thrown upon this assumed identity, until but a few years ago it was definitely shown to be in error, and today the vitamins are again considered to be distinct. However, for thirteen years or more a large amount of experimental work was being carried out on a working hypothesis that had been insecurely based upon negative evidence Today much of this work is unintelligible and represents almost a dead loss The unfortunate part of this incident is that the lesson it should teach has not been perceived as generally as its importance justifies. Today many investigations are being conducted on the properties and distributions of these two components of the so-called "B complex" on the tacit assumption that no other components exist. If other components are later definitely characterized a majority of these studies also will be uninterpretable.

The third possible interpretation of negative experimental data is the greatest obstacle to the formulation of definite conclusions, because important biological facts may be obscured for reasons that may not be divulged by methodical experimentation. Investigations in the metabolism of creatine and creatinine are replete with illustrations of biochemical transformations that seem to occur only under severely circumscribed conditions the nature of which is not clear at the present time. In some cases, the nature of the obscuring conditions has been revealed. Only recently a group of investigators failed to obtain evidence that carotin could replace vitamin A

in nutrition and on this negative evidence they concluded that carotin was incapable of being converted into vitamin A. They explained the positive results obtained in other laboratories by showing that the carotin used there was not of the highest purity and by assuming that in all probability either it or the natural oils in which it was dissolved contained traces of vitamin A. More recently, however, they have reported that the error was in their own technic In the first experiment they dissolved their carotin before administration to their rats in ethyl oleate, which they have since found undergoes a spontaneous oxidation in the course of which any carotin or vitamin A dissolved in the oil is destroyed. When highly purified carotin is given to the experimental rats dissolved in ethyl laurate, daily doses as small as .005 gram induced an appreciable gain in weight.

As a final illustration of the existence of conditions that obscure biological relations and prevent the securing of positive experimental results, the effect of sunlight on calcium deposition may be mentioned. In studies of calcium metabolism, no relation is demonstrable within wide limits between the calcium-to-phosphorus ratio in the food and the extent of calcification in the bones if the experimental animals have access to sunlight. In other words, negative results may be obtained. That these negative results do not mean that no relation exists would be evident when the experiments are repeated under conditions such that the animals have no access to direct sunlight.

This discussion of the interpretation of negative evidence does not imply that such evidence is valueless. In many cases it may be significant, but in all cases its interpretation should call for the exercise of extreme caution, particularly if other experiments have yielded good evidence possessing a contrary significance. The law of organic evolution cannot be overturned by the failure of the known facts and the theories of genetics to explain it, since evolutional transformations may depend on causes that have eluded even the precise and methodical experimentation of geneticists. Nor can it be said even that the Lamarckian theory of the inheritance of acquired characters can ever be disproved by showing that, in specific instances, acquired characters are not inherited.

In the best controlled experiments, unaccountable variation in measurements obtained under as nearly as possible the same conditions will always occur. This unaccountable variation, representing the experimental error, will always tend to discount to some extent the significance of differences in measurements obtained under different conditions and thus to

obscure the very effects it is desired to observe. In drawing any conclusion as to the existence or the magnitude of these effects, some fair allowance must be made for the influence of the unaccountable variation in measurements, representing uncontrolled or imperfectly controlled conditions. This is the function of statistical or biometrical methods, and their value in the interpretation of the data of biological experiments is difficult to over-emphasize.

In recent years an active development of biometrical methods has occurred and an application of them has been made to other problems than the mere calculation of probable errors, coefficients of correlation, etc. They may now be used to unravel, sometimes in a fairly satisfactory manner, the tangled and otherwise unintelligible data of imperfectly controlled experiments. This is all very fine, in that it permits the extraction from an experiment of the last particle of its information; but a real danger lies in the situation in so far as the existence of such methods of dealing with poorly controlled experiments may constitute an enticing invitation to slovenly experimental work. Suffice it to say that any experimental method that avoids a source of error is far to be preferred to another that trusts to probabilities for its elimination.

It should not be inferred, however, that the use of statistical methods in interpreting the results of a given experiment is a confession that the experiment was poorly controlled, though this inference is not infrequently made. Statistical methods have been put to greater use in the physical sciences where the control of experimental conditions can be much more complete, than in the biological sciences. In fact, the latest investigations of physicists concerning the fundamental nature of the universe indicate that the laws of probability, upon which statistical methods are based, are of more fundamental significance even than the law of causation. When the behavior of individual atoms or electrons is observed, it is found impossible to predict with certainty their positions at any given time. Determinism does not exist in events in which atoms and electrons are singly involved. But when atoms and electrons move in crowds their behavior is calculable by the laws of probability. In the same way, the throw of a single penny is quite unpredictable, but if we throw a million tons of pennies, we can predict that 500,000 tons of pennies will show heads and 500,000 tons will show tails. This illustration is borrowed from Sir James Jeans and concerning it he says:

"We may be tempted to instance it as evidence of the uniformity of na-

ture, and to infer the action of an underlying law of causation· in actual fact it is an instance only of the operation of the purely mathematical laws of chance."

Yet the number of pennies in a million tons "is nothing in comparison with the number of atoms in even the smallest piece of matter with which the earlier physicists could experiment. It is easy to see how the illusion of determinacy—if it is an illusion—crept into science."

H. H. M.

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